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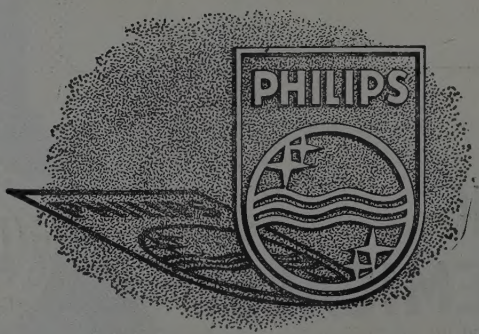

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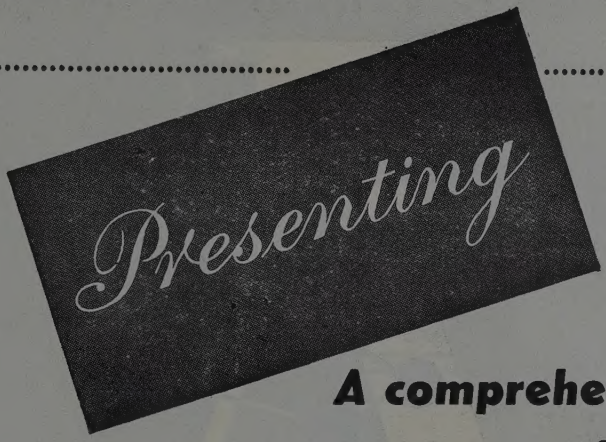

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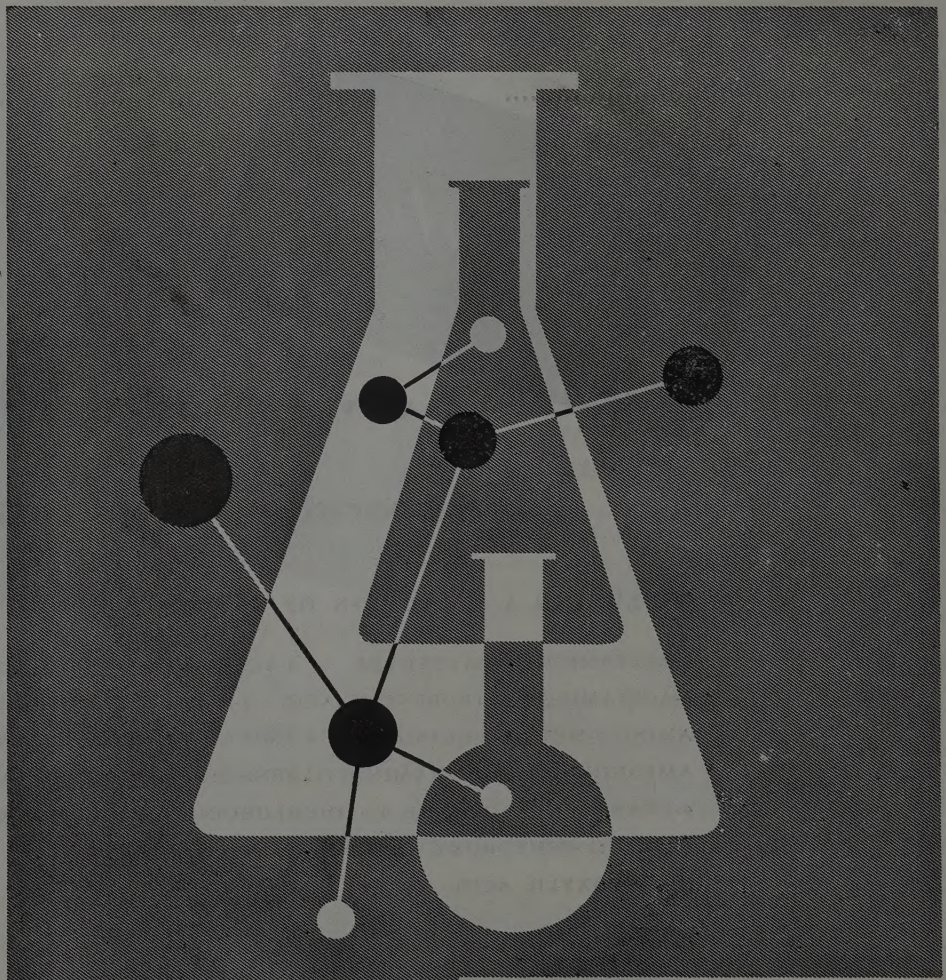

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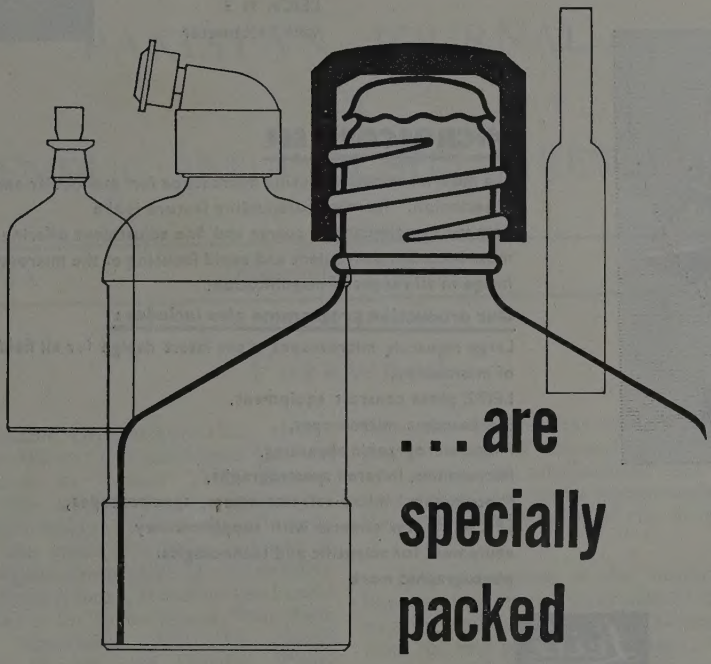

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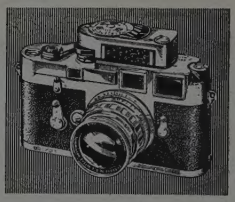

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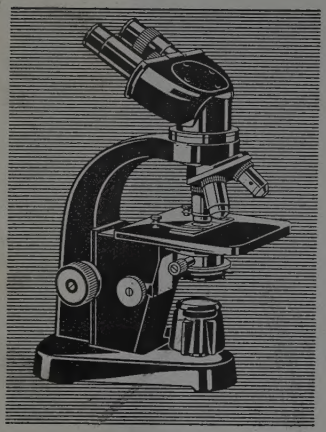

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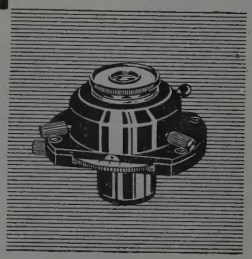

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Foreword

It is a remarkable circumstance that, alongside of modern medicine, the mediaeval Graeco-Arab system known as 'Unani Tibb' (Ionian medicine), and the ancient healing system of Ayurveda continue to hold the field in this subcontinent, catering to the needs of a large majority of the population. Quite irrespective of the validity of the theories of these systems, it was realised early that there was still a lot to be learnt from their pharmacopoeial experience, and the classic work of Dymock, Hooper and Warden, 'Pharmacographica Indica,' published about the turn of the century, is a pointer in that direction. In the 1920's a concerted effort was made at the School of Tropical Medicine in Calcutta, towards the pharmacological investigation of the active constituents and isolates of a whole series of plant drugs. Subsequently, as a more purposeful approach from the point of view of the indigenous systems of medicine, a drug research institute was established at the Ayurvedic and Unani Tibbi College in Delhi, on the initiative of the late physician and statesman, Hakim Ajmal Khan. The very first drug that came up for investigation at this institute was *Rauwolfia serpentina*, which was systematically used over a long period by the late Hakim Ajmal Khan in the treatment of a variety of mental ailments, in the form of a powder with a rather appropriate name 'Dawa-ush-Shifa' (the healing draught). Following the communication on the now well known ajmaline series of alkaloids, increasing interest has been shown in the therapeutic virtues of *Rauwolfia*, and many groups of workers in the subcontinent and the world over have taken part in the various aspects of studies in the active constituents of the drug.

It is a measure of the importance *Rauwolfia* has acquired in the treatment of hypertension and as a tranquilliser in mental ailments, that starting from the first publication in 1931, the bibliography on the subject already counts over two thousand references. These references also include reviews and articles presenting the subject matter from various standpoints and interests. In the present special issue of this journal, it is not intended to cover all that ground, but rather to record the more recent contributions to studies in *Rauwolfia* carried out under the Pakistan Council of Scientific and Industrial Research. Aside of studies in the alkaloidal complexes isolated from fresh undried material, this issue also reports the results of work on the cardiac activity of one of the complexes, namely, serpajmaline, and its correlation with the chemical structure of its constituent alkaloids.

It is hoped that the material presented in this issue will stimulate further work in the field of *Rauwolfia* alkaloids, and that the bibliography included in it will also be helpful to that end.

S.S.

STUDIES IN THE ALKALOIDS OF RAUWOLFIA SERPENTINA, BENTH. AND THE MODE OF THEIR OCCURRENCE

SALIMUZZAMAN SIDDIQUI, S. A. WARSI AND M. ALAUDDIN

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From the roots of *Rauwolfia serpentina, Benth.*, obtained from the Bihar province of India, S. Siddiqui and R.H. Siddiqui[1] isolated in 1931* a series of crystalline bases, namely, ajmaline, ajmalinine, ajmalicine, serpentine, and serpentinine, taking advantage of the differences in their basic strength on the one hand, and the solubilities of their hydrochlorides on the other. Later, in 1939, working on the roots and root bark of the plant, obtained from the more temperate climate of the Dun Valley, S. Siddiqui[2] reported the isolation of two isomers of ajmaline, namely isoajmaline and neoajmaline, a base melting at 220°C. and, from the neutral fraction of the alcoholic extract, a white crystalline alkaloid melting at 234°C., in a yield of 0.1%. From this last mentioned base and its mother liquors it was subsequently possible through repeated fractional crystallisations from methanol and acetone to isolate a crystalline substance, which melted at 270-273°C., and yielded on mild hydrolysis an acid and a base. These findings, however, were not reported, awaiting further work on the problem which had to be held over during the War and its aftermath. In 1952, Schlittler et al.[3] reported the isolation of this base under the name, reserpine, from the so-called 'oleo-resin' fraction which actually corresponds to the 'neutral fraction' referred to above. These authors further claimed reserpine as the sole therapeutic agent in the dual role of its sedative action on the one hand, and blood pressure reducing property on the other. This claim had to be somewhat modified later on, particularly with the isolation of rescinnamine, which has similar pharmacological action.[4] The isolation of this and other minor bases subsequently reported over the years by various authors and listed in Table 1 has not however, materially affected the position claimed for reserpine in respect of its therapeutic action.

Against the background of earlier work briefly referred to above and also taking into account the conflicting pharmacological and clinical findings of various groups of workers in respect of the individual alkaloids and preparations of *Rauwolfia serpentina*, it was considered of interest to isolate and study the various alkaloidal factors as far as possible in the form they occur in the plant body. Moreover, as stocked samples of *Rauwolfia* roots were found to give comparatively low yields of crystalline bases, and profiting from the observation that the crystalline constituents isolated from fresh plant materials through dialysis with solvents can get completely destroyed on drying,[8] apparently due to enzymic and oxidative degradations, fresh undried roots of *Rauwolfia serpentina* were employed for this study.

Working on fresh roots harvested from an experimental plantation in East Pakistan, a procedure was evolved and briefly reported by Siddiqui for the isolation of a number of alkaloidal complexes,[9] on the basis of their varying solubilities in polar and non-polar solvents and expressly avoiding the use of acids, alkalies and other chemical reagents. This procedure which is described in detail in the experimental, consists in dialysing undried roots chopped into 2-3 cm. lengths with alcohol, removing the solvent from the combined dialysates, and resolving the semisolid residue into the following fairly well-defined alkaloidal factors, on the basis of their respective solubility in petroleum ether, ethyl acetate, amyl alcohol and water:

(1) A petroleum ether soluble, greenish viscous liquid, which has been named 'resajmaline' mainly consisting of fatty matter, serposterol and other unsaponifiables. It contains 2.3% reserpine, 0.5% rescinnamine, some of the other weaker bases, and traces of ajmaline (yield 0.5-0.8%).

(2) An ethyl acetate soluble, petroleum ether and water insoluble, alkaloidal complex, named 'ajmalexine' which forms a cream coloured powder consisting mainly of the alkaloids of the ajmaline group, and the weaker *Rauwolfia* bases including 5.5% of reserpine and 2.5% rescinnamine (yield 0.2–0.3%).

(3) An ethyl acetate insoluble, amyl alcohol soluble factor, designated as 'serpajmaline' which forms a light cream coloured powder and is readily soluble in water. It is chromatographically free from reserpine and other weaker bases of the group, and mainly consists of the alkaloids, ajmaline, serpentine and serpentinine, along with two unknown substances giving a greenish and an orange fluore-

* A year later, in 1932, van Itallie and Steenhauer[5] reported the isolation of a base from *Rauwolfia serpentina* which was obviously identical with ajmaline, but they named it as rauwolfine. This has been responsible for a double confusion in nomenclature, due to the added fact that the isolation of a base with that name was also reported in the same year by Koepffli as a constituent of *R. caffra*.

scence. Besides these three fairly well-defined fractions, which account for about 70% of the total assayed alkaloidal content, a comparatively small quantity of an ill-defined light brown product partially soluble in water and aqueous mixtures of amyl alcohol is also obtained in this working along with a reddish, syrupy, water soluble ballast containing about 5–10% of the residual alkaloids.

Apart from protecting the bases against aerial oxidation and enzymic degradation, a further advantage of working with freshly harvested material lies in the fact that the cell walls of the fresh roots virtually act as a semi-permeable membrane, retaining a substantial portion of the non-alkaloidal ballast, and the dialysates thus yield on removal of the solvent only 5% of a semisolid matter on air dried basis, as against 10–12% extractive from the alcoholic percolates of the air dried powdered roots, the non-alkaloidal ballast which greatly complicates the isolation work being thus reduced by about half.

In contrast to the rather long drawn out procedures which had to be adopted by Siddiqui and Siddiqui,[1] and later by Robinson et al.,[10] and other workers for the isolation of alkaloids from the alcoholic extracts of dried powdered roots, the complexes referred to above have served as convenient source materials for the various alkaloids, following a comparatively smooth and simple procedure described for their isolation in the experimental. Apart from ajmaline, neoajmaline, serpentine, serpentinine and reserpine, a base melting at

TABLE 1.—ALKALOIDS FROM *Rauwolfia serpentina*.[7]

Name	Empirical formulae	M. p. °C.	First isolated by
Ajmaline	$C_{20}H_{26}O_2N_2$	158 - 160	Siddiqui & Siddiqui (1931)
Ajmalinine	$C_{20}H_{26}O_3N_2$	180 - 181	,, ,, ,,
Ajmalicine		250 - 252	,, ,, ,,
o-Yohimbine	$C_{21}H_{24}O_3N_2$	258 - 259	Weisenborn et al. (1954)
Raubasine (Alkaloid II) ..	,,	247 - 248	Popelak et al. (1953)
Alkaloid F	,,	253 - 254	Neuss et al. (1954)
Tetrahydroserpentine ..	,,		Bader & Schwarz (1952)
Serpentine	$C_{21}H_{20}O_3N_2$	157 - 159	Siddiqui & Siddiqui (1931)
Serpentinine	$C_{21}H_{22}O_3N_2$ (?)	263 - 265	,, ,, ,,
Isoajmaline	$C_{20}H_{26}O_2N_2$	264 - 266	Siddiqui (1939)
Neoajmaline	$C_{20}H_{26}O_2N_2$	205 - 207	,, ,,
Rauwolfinine	$C_{19}H_{26}O_2N_2$	235 - 236	Chatterjee & Bose (1951)
Reserpine	$C_{33}H_{40}O_9N_2$	277 - 278	Mueller et al. (1952)
Raupine .. (Identical)	$C_{19}H_{22}O_2N_2$	325	Bodendorf & Eder (1953)
Serpagine ..		over 300	Stoll & Hofmann (1953)
Reserpinine ..		238 - 239	Schlittler et al. (1954)
Alkaloid A ..		243 - 244	Neuss et al. (1954)
Alkaloid C .. (Identical)	$C_{22}H_{26}O_4N_2$	240	Hofmann (1954)
Raubasinine (Alkaloid I) ..		228	Popelak et al. (1953)
Corynanthine (Rauhimbine) ..	$C_{21}H_{26}O_3N_2$	218 - 225	Hofmann (1954 & 1954)
Isorauhimbine	$C_{21}H_{26}O_3N_2$	225 - 228	Hofmann (1954)
Yohimbine	$C_{21}H_{26}O_3N_2$	234 - 236	Bader et al. (1954)
Rescinnamine	$C_{35}H_{42}O_9N_2$	226	Haack et al. (1954 & 1955)
		238	Klohs et al. (1954)
3-epi α-yohimbine ..	$C_{21}H_{26}O_3N_2$	125 - 128 / 181 - 183	Bader et al. (1954)
Serpine	$C_{21}H_{26}O_3N_2$	213	Chatterjee & Bose (1954)
Serpinine	$C_{20}H_{24}ON_2$ or $C_{21}H_{26}ON_2$	315 - 317	Bose (1955)
Thebaine	$C_{19}H_{21}O_3N$	195	Hofmann (1954)
Papaverine	$C_{20}H_{21}O_4N$	147	,, ,,
Reserpiline	$C_{23}H_{28}O_5N_2$	amorphous	Klohs et al. (1954)

300-310°C. and probably identical with serpinine[11] has also been isolated in the present working.

Several batches of *Rauwolfia serpentina* roots harvested from the plantation in East Pakistan at various age levels were worked out according to this procedure with the results tabulated in the experimental (Table 2). In the course of this work it has been noted that the maturity of the roots has a considerable bearing on the yields of the various complexes. In one of the batches harvested at the end of July, very little of the fraction corresponding to ajmalexine could be isolated. Moreover, the age of the roots was also found to influence the alkaloidal content of the serpajmaline complex. Alkaloidal content of this complex was found to be about 35% in comparatively young roots and nearly 50% in the roots of about 2 year old plants.

The seasonal variations seemed to be even more significant. The dialysates of the roots harvested during the summer months, May-July, on partitioning between water and ethyl acetate did not completely divide up between these two solvents, leaving some quantity of an insoluble middle layer, which seemed to increase with the age of the roots. The roots harvested in January (about 2 years old) did not give such a middle layer under parallel conditions of working. Moreover, only neoajmaline was obtained from serpajmaline in this working while all the batches received during the summer months gave the normal isomer, ajmaline. This finding recalls the earlier observation of Siddiqui[8] that neoajmaline is formed under more temperate climatic conditions, as the principal alkaloidal constituent of *Rauwolfia*.

The fact that the major part of the total alkaloids occurs in the form of 'complexes,' rather than as simple salts or in the free state, is fairly evident from the fact that ajmalexine is insoluble in water or dilute acetic acid, but on breaking up the complex with alkali, the liberated bases readily dissolve in dilute acids. Again, in contrast to all the *Rauwolfia* alkaloids and their simple salts, the bases contained in resajmaline are readily soluble in petroleum ether, and cannot be extracted from the ethereal solution with the help of dilute acetic or mineral acids, which should point to their occurrence in the form of a petroleum ether soluble complex. The solubility of serpajmaline in amyl alcohol also leads to the same conclusion.

On the basis of fairly exhaustive studies in the pharmacology of serpajmaline,[12] which is obtained in an yield of 1.3-1.6% on dry weight basis, it has been found that it is highly potent in its hypotensive activity, without the therapeutically complicating sedative and central depressant action characteristic of reserpine and other *Rauwolfia* preparations. It has been further noted that serpajmaline has a strong antifibrillant action on the isolated heart of the rabbit and the dog.[13] On the other hand, it has very low toxicity, dosages as high as 1000 mg./kg. body weight proving non-fatal in mice.

In order to determine the role of its non-alkaloidal constituents in this cardiac action of serpajmaline, its carbohydrate component was isolated free from basic impurities, employing mildest possible methods. A nearly colourless, treacly, nitrogen-free, water soluble product thereby obtained in an yield of about 15% on the weight of the complex, was found to have the same order of antifibrillant action as serpajmaline and ajmaline. None of its individual constituents, however, quantitatively account for the total activity of the serpajmaline complex, which would appear to be derived from the mutually potentiating, synergistic action of the various alkaloidal and non-alkaloidal components of the complex. Further work on problems arising out of the findings recorded in the present paper is in progress.

Experimental

The procedure employed for obtaining the various alkaloidal complexes was basically identical with that briefly described earlier by Siddiqui.[9] Sixteen kg. of fresh undried roots of *Rauwolfia serpentina* (corresponding in dry weight to 7.2 kg.) were harvested when 21 months old from an experimental farm in East Pakistan, flown to Karachi and immediately on receipt cut into 2-3 cm. lengths and soaked in ethanol. The extract was removed after every 48 hrs. About 20 litres of ethanol (95%) was needed each time to soak the chopped roots. Each extract after removal was first concentrated in a cyclone evaporator to about 5 litres and finally *in vacuo* to a treacly mass, not allowing the temperature to exceed 50°C. The weights of the liquidish residues obtained for the 5 percolates were 750, 600, 75, 68, and 61 g., respectively. A further extraction was carried out with 80% ethanol and the semisolid mass obtained was 135 g. The extraction was considered complete as a subsequent extract with 80% ethanol gave a very small amount of the semisolid residue.

The roots were initially assayed for their alkaloidal content and then after the withdrawal of each percolate:

Initial	2.04%	After ext. IV	0.15%
After ext. I	0.99%	,, V	0.12%
,, II	0.55%	,, VI	0.06%
,, III	0.49%	,, VII	0.03%

The still liquidish residue (about 2 litres) was stirred up with water (2 litres) and shaken with ethyl acetate when the extract divided up into ethereal and aqueous layers. The lower aqueous layer was exhaustively extracted with ethyl acetate (200 ml. each time, total 1 litre) until the ethyl acetate layer was only faintly yellow. The combined ethyl acetate extracts were washed with water (100 ml., 3 times) and the aqueous washings added to the main aqueous extract.

Isolation of Serpajmaline.—The aqueous extract was repeatedly shaken out with amyl alcohol (250 ml. each time, total 5 litres) till further extractions gave only negligible quantities of residue on removal of the soevent from an aliquot fraction. The combined amyl alcoholic extract was shaken out repeatedly with water (300 ml. each time, 5 times). The aqueous extracts on complete removal of the solvent *in vacuo* below 50°C. and subsequent drying yielded a light cream coloured powder (97 g.).

The amyl alcohol was then concentrated to about half the volume *in vacuo* and again exhausted with water. The aqueous extracts were shaken out with ethyl acetate, and on complete removal of water *in vacuo* yielded a further quantity of light cream coloured residue (18 g.), making for a total yield of 115 g. (1.59%).

Separation of Resajmaline and Ajmalexine.—The combined ethyl acetate extracts were freed of the solvent *in vacuo* and to the residue (80.8 g.) enough petroleum ether was cautiously added to adequately cover the residue without stirring, and was carefully decanted off after every 48 hrs. Further extractions were similarly carried out with petroleum ether until a colourless extract was obtained, leaving practically no residue on removal of the solvent.

Resajmaline.—The petroleum ether extract on complete removal of the solvent yielded a green thick oily product (55 g., 0.76%).

Ajmalexine.—The petroleum ether insoluble residue on drying *in vacuo* gave a light cream coloured powder (24 g., 0.33%) on the weight of the dry roots.

Several batches of fresh *Rauwolfia* roots obtained from the experimental farm were worked according to the process detailed above and gave the various complexes in yields indicated in Table 2.

Serpajmaline and its Constituent Bases.—Serpajmaline is a light cream coloured amorphous powder readily soluble in water, absolute alcohol and moist acetone, insoluble in benzene, ethyl acetate, ether and other non-miscible solvents. It begins to soften at 85°C., sticks to the sides at about 100°C., and froths up around 130°C. The aqueous solutions of serpajmaline show a pH of 5-6.

Attempts were initially made to separate the stronger, yellow bases from the comparatively weaker colourless bases of the ajmaline group by extracting with immiscible solvents after adjusting the pH of the aqueous solution to 7 with dilute ammonia. This procedure of isolation, however, did not work as the complex does not seem to break at this pH.

Serpajmaline (5 g.) was treated with cold dilute 10% ammonia (2 ml.) and the yellow granular precipitate was filtered and washed with ice-cold water under suction. The aqueous filtrate was extracted thrice with ethyl acetate (15 ml.). The precipitate (2 g.) was taken up in the ethyl acetate washings when practically the whole of it went into solution leaving a negligible quantity of a dark

TABLE 2.—YIELDS OF *Rauwolfia* ALKALOIDAL COMPLEXES FROM DIFFERENT BATCHES OF FRESH ROOTS.

Harvesting period	Age	Fresh roots kg.	Calculated dry weight kg.	Serpajmaline %	Ajmalexine %	Resajmaline %
End of January 1958 ..	15 months	18.0	9.0	1.55	0.22	0.833
Second week of July 1958 ..	10½ „	14.5	8.12	1.13	0.244	0.532
End of July 1958 ..	11 „	16.5	9.075	1.123	—	0.462
First week of October 1958..	13 „	17.0	7.65	1.2	0.29	0.60
End of May 1959 ..	21 „	16.0	7.20	1.6	0.33	0.76

brownish insoluble residue. The moist ethyl acetate extract was cooled in ice and saturated with carbon dioxide, finally with the addition of petroleum ether (about 10 ml.) whereby the stronger, yellow bases were removed as carbonate, leaving a faint yellow solution. After filtration and drying over anhydrous sodium sulphate the solvent was removed *in vacuo*, the residue (1 g.) extracted with benzene and the benzene soluble fraction (0.77 g.) after removal of the solvent was crystallised from methanol. The colourless crystallisate thus obtained was identified as ajmaline. The benzene insoluble fraction on crystallisation with methanol gave colourless long needles, subliming at 300-310°C., and probably identical with serpinine[11] (m.p. 315°C.). The yellow sticky precipitate of the carbonates was completely freed of the solvent *in vacuo*, dissolved in the least quantity of 10% acetic acid, charcoaled and treated with an ice-cold aqueous solution of potassium iodide. The resulting yellowish precipitate was filtered, taken up in methyl alcohol (8-10 ml.) and kept in the cold, when glistening yellow rods of serpentine hydriodide crystallised out (m.p.278°C., decomp.). The alcoholic filtrate was again treated with activated charcoal, slightly concentrated and carefully basified with dilute caustic soda, when serpentinine almost immediately came out in lemon yellow crystals which after washing with cold dilute methanol melted at 264°C., and showed no depression in m.p. on admixture with an authentic sample of serpentinine.

In a subsequent working, the basic fraction obtained on treatment of a cold aqueous solution of serpajmaline with ammonia was directly separated into a benzene soluble and an insoluble fraction avoiding the use of ethyl acetate. A little petroleum ether was added to the benzene solution when on saturation with carbon dioxide it became almost colourless. The residue on removal of the solvent was crystallised from methanol yielding ajmaline. The precipitated insoluble matter along with the benzene insoluble fraction containing the yellow bases could then be worked as described above for the isolation of serpentine and serpentinine.

Serpajmaline complex obtained from the roots harvested at the end of January yielded only neoajmaline melting at 205-207°C.

Ajmalexine.—It is a light cream coloured amorphous powder soluble in ethyl acetate, mostly soluble in benzene fairly so in ethanol and methanol and insoluble in petroleum ether. No quite satisfactory method of isolating the individual bases from this complex could be evolved owing to the fact that the complex connot be effectively broken without treatment with stronger alkali which results in the partial hydrolysis of the weaker bases like reserpine and rescinnamine. For the isolation of reserpine, ajmalexine (6 g.) was dissolved in hot ethyl acetate (25 ml.), and the solution after quickly drying over sodium sulphate was adsorbed over a column of silica gel (E. Merck, for partition chromatography), 25 cm. long and 2 cm. in diameter. The column was first eluted with ethyl acetate until the eluate became colourless and then exhausted with methyl alcohol. The ethyl acetate extract was freed of the solvent *in vacuo*, the residue taken up in hot benzene and cooled, and the small quantity of the greenish precipitate which deposited was filtered off and added to the methanol extract. The benzene soluble fraction on removal of the solvent *in vacuo* was dissolved in a small quantity of methanol and kept in the cold when colourless crystals (0.19 g.) melting at 268-271°C., were obtained and identified as reserpine. The methanolic eluate and the mother liquor of reserpine which gave positive HNO_3 colour reaction for ajmaline were not followed for the isolation of other alkaloidal constituents for the present.

Resajmaline.—The separation of individual alkaloidal components from this lipoid soluble complex also presented many manipulative difficulties. In one of the workings, resajmaline (50 g.), obtained from comparatively young roots harvested in February, was taken up in a mixture of ether, petroleum ether and methanol, and kept in the cold. The crystalline deposit thereby obtained was repeatedly crystallised from a mixture of acetone and methanol when it finally gave colourless silky needles m.p. 155-160°C., identified as serposterol. The dark green filtrate from the original crystallisate was extracted with a mixture of methanol and dilute 10% aqueous ammonia (50:50). The petroleum ether layer was freed of the solvent *in vacuo* and was not pursued further for the present. The ammoniacal extract was acidified with dilute acetic acid to pH 6 and the solvent removed *in vacuo*. The residue gave through ethyl acetate a crude crystallisate, the ether soluble fraction of which on repeated crystallisation from methanol yielded colourless needles (m.p. 205°C), identified as neoajmaline. The ether insoluble portion of the crude crystallisate gave from methanol, on repeated crystallisation, colourless needles m.p. 250-254°C., identified as ajmalicine, by taking mixed melting point with an authentic sample.

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STUDIES IN THE AJMALINE SERIES

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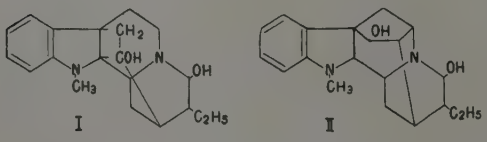
Out of the various bases isolated by Siddiqui and Siddiqui[1] from the roots of *Rauwolfia serpentina*, ajmaline, the principal alkaloid, was subjected by the authors to a more detailed study.[2] In the course of this study, it was found that ajmaline contains one N-methyl group, forms neutral nitrosoamine derivative and yields a tertiary methyl base through its methiodide. A secondary character was, therefore, concluded for one of its two nitrogen atoms. The presence of an olefinic double bond in the molecule was deduced from the fact that it took two atoms of bromine in the cold, giving a product which when crystallised from alcohol melted at 230°C. (decomp.) and analysed for dibromoajmaline. On the basis of their further studies in the chemical characteristics of ajmaline and the colour reactions of the zinc dust distillation products, already reported in the meanwhile by van Itallie and Steenhauer,[3] Siddiqui and Siddiqui further suggested a structure for ajmaline closely allied to strychnine.

Following these investigations, Robinson and his associates[4,5,6,7], carried out comprehensive studies in the constitution of ajmaline and assigned to it the structural formula I which was later slightly modified by Woodward as II.[8]



In their first communication on the subject, Robinson et al. generally confirmed the earlier findings of Siddiqui and Siddiqui, but disagreed with the interpretations they put forward for some of them. These points of disagreement may be summarised as follows:—

1. Dibromoajmaline is actually bromoajmaline hydrobromide, as its aqueous solution gave precipitate on the addition of an aqueous solution of silver nitrate, and monobromoajmaline on liberating the base with ammonia.

2. Ajmaline is di-tertiary and not secondary-tertiary in character, as it yields a quaternary base through its methiodide, and the action of nitrous acid on ajmaline hydrochloride in aqueous solution gives a basic C-nitroso and not N-nitroso derivative.

3. Neoajmaline reported by Siddiqui[9] could not be distinguished from ajmaline with any certainty as its hydrochloride gave ajmaline hydrochloride on recrystallisation, and the m.p. 205-207°C. recorded for neoajmaline as against 158-160°C. for ajmaline could be considered as that of anhydrous ajmaline.

The isolation of serpajmaline and other therapeutically significant alkaloidal complexes by Siddiqui[10] from the fresh undried roots of *Rauwolfia serpentina*, followed by the observation that the serpajmaline complex besides being hypotensive in character[11] also possessed antifibrillant action[12] on the isolated heart of the rabbit and dog, revived fresh interest in the chemistry and pharmacology of the alkaloidal constituents of *Rauwolfia*. Studies in this direction led to the finding that ajmaline, the main alkaloidal component of serpajmaline, has the same order of antifibrillant action as the complex as a whole and that serpentine also has this character although to a lesser degree, while serpentinine is inactive. In view of these findings, it was considered of interest to study the relationship between the chemical constitution of ajmaline and its cardiac action. As a result of this study it has been found that the reduction of the benzene ring in ajmaline (hexahydroajmaline) lowers its cardiac activity to about 1/1000. On the other hand, methyl-, acetyl- and dihydro-ajmaline, in all of which the benzene ring remains intact, were found to be devoid of any cardiac activity,[13] establishing the fact that the dynamic isomerism of the carbinolamine and imine-aldelyde structure, $=N.\overset{|}{C}H(OH) \rightleftharpoons =NH \quad \overset{|}{C}HO$, serving as what may be described as a 'cardiophoric' grouping, is essential for the cardiac action of the ajmaline molecule, and that the benzene ring in it has only a potentiating action.

In the course of these investigations the earlier work of Siddiqui and Siddiqui (loc. cit.) on ajmaline was repeated and their conclusions re-examined in the light of disagreement expressed by Robinson et al. and referred to at the outset.

As a result of this re-examination the position may be stated as follows:—

The bromination product obtained on titration

of ajmaline with bromine in chloroform solution melts, as reported by Robinson et al. at 295 °C. (decomp.), but on crystallisation from alcohol it shows m.p. 235-239 °C. as recorded by Siddiqui and Siddiqui. That this variation in melting point is not due to the anhydrous state of the former is evidenced from the fact that on drying the bromo-product crystallised from alcohol *in vacuo* at 100 °C., the melting point is not affected. In case of iso-ajmaline also the melting point recorded by Robinson for its bromination product is 288-289 °C. (decomp.) but it has been found that on crystallisation from methanol it melts at 225-228 °C.

Further, it has now been noted that, when the drop by drop addition of bromine in chloroform solution is carried on till there is a persistent turbidity, which occurs after the addition of only 1.7 atoms of bromine, and the addition product is worked up as described in the experimental, a bromo derivative is obtained which on crystallisation from methanol melts at 178-182°C. (decomp.), the melting point remaining unaffected on further crystallisations or drying *in vacuo* at 100 °C. These findings suggest the formation of three isomeric bromo derivatives, of which the one melting at 296 °C. is an unstable form.

The observations made in the course of the bromination studies, which were extended to a number of other derivatives of ajmaline, would appear to indicate that the carbinol amine tautomeric grouping induces a potential olefinic double bond in the complex ring system of ajmaline, presumably at the expense of the bridge ring provided in its structure by both Robinson and Woodward. This could account for the formation of a chloroform soluble, dibromo addition product from which HBr is secondarily eliminated, yielding a monobromo, chloroform insoluble hydrobromide, which on treatment with aqueous ammonia yields monobromoajmaline. This view gets a measure of support from the fact that dihydroajmaline fails to give a bromo derivative, and that secondary elimination of HBr from bromine addition products of bases containing an olefinic linkage has been reported earlier, e.g. in the case of the conessine series of alkaloids.[14] The fact that hexahydroajmaline is saturated to bromine would only show that the reduction of the benzene ring stabilises the bridge ring, rather than constitute an argument in favour of a straightforward bromine substitution reaction at the aromatic centre, under the experimental conditions of the reaction.

Again, methylajmaline, in which the carbinol-amine grouping is eliminated and the aldehyde group stabilised, takes up nearly 3 atoms of bromine, giving an amorphous powder which melts at 290 °C., is difficultly soluble in water and gives a bromine-free product on liberation with aqueous ammonia, which could be identified with methylisoajmaline through its hydriodide (m.p. 195 °C.; methylajmaline hydriodide, m.p. 129-131 °C.). The formation of such a loose bromo compound might be explained by the enolisation of the aldehydic carbonyl of the base from which bromine is completely eliminated on treatment with ammonia, methylajmaline being converted into methylisoajmaline in the process.

In re-examining the alternatives of secondary or tertiary character of one of the two nitrogen atoms of ajmaline, the findings of Siddiqui and Siddiqui in regard to the formation of a neutral, N-nitroso derivative of ajmaline on treatment of the base with nitrous acid in acetic acid medium, and of a tertiary methyl base through the methiodide, have been repeatedly checked up and confirmed. It has, however, been noted that, as reported by Robinson et al., treatment of ajmaline in hydrochloric acid medium gives a C-nitroso basic product. The resolution of this point of disagreement, however, had in effect already emerged from a subsequent communication of Robinson et al. (loc. cit.) which formulated the tautomeric carbinol-amine and imine-aldehyde structure for ajmaline. In this context, the authors had concluded from their studies that ajmaline in neutral or acid soluti on contains $=N.\underset{|}{C}H(OH)$ 'the abnormal carbinol-amine group', but that in alkaline solution only a small proportion of the form $=NH\ \underset{|}{C}HO$ (aldehyde-imine) is probably present in equilibrium with the carbinol-amine. The fact that the N-nitroso derivative of ajmaline is smoothly obtained in nearly theoretical yield in acetic acid medium controverts such a conclusion.

Siddiqui and Siddiqui had concluded a mono- and a di-acid character for ajmaline and isoajmaline, respectively. This conclusion proved erroneous, because isoajmaline hydrochloride had been prepared by them in ethereal medium, and ajmaline hydrochloride by reacting the base with aqueous hydrochloric acid, in which the weakly basic nitrogen in both the bases fails to form a salt.

With regard to neoajmaline which Robinson et al. have considered to be probably anhydrous ajmaline, it would appear from the present study that the two bases are quite distinct isomers. In the course of isolation of the alkaloidal complexes and individual bases from the fresh roots of *Rauwolfia*, it was observed that with identical procedure of isolation some batches of the drug yielded only neoajmaline while others gave the normal isomer,

ajmaline, depending on the age of the roots harvested from the same experimental farm. Moreover, neoajmaline was repeatedly obtained by slowly adding a warm aqueous solution of the hydrochloride to a strong, well-cooled solution of ammonia, and crystallising the precipitated base from dilute methanol. These conditions would not favour the formation of an anhydride, the more so because as reported by Siddiqui and Siddiqui the water of crystallisation tenaciously adheres to the ajmaline molecule, being completely eliminated only on heating *in vacuo* over a long period at 150°C. It has, however, been observed that the reduction of neoajmaline and ajmaline with borohydride results in the production of identical dihydroajmaline. The isomerism may, therefore, be due to the spatial arrangement of the H and OH of the carbinol amine group.

Taking into account the behaviour of ajmaline and its derivatives in respect of their cardiac action and the abnormal character of the carbinol-amine grouping in ajmaline, it was considered of great interest to study the stability of the carbinol-amine ring of the base, and the possible bearing of this factor on its cardiac action.

With this object in view, the von Braun reaction of cyanogen bromide[15] was carried out with ajmaline and hexahydroajmaline, after protecting the reactive hydroxyl groups and stabilising the carbinol-amine structure through acetylation. Under the conditions described in the experimental, a monocyano-monoacetyl derivative was readily obtained on treatment of diacetylajmaline with cyanogen bromide, while the same reaction takes about 24 hours for completion with hexahydrodiacetylajmaline. The comparative speed with which this reaction proceeds is a measure of the instability of the carbinol amine ring in ajmaline, and its considerable stabilisation through the reduction of the benzene ring, offering a co-relation of the stability of this ring with the intensity of cardiac action which, as referred to above, is 1000 times lower for hexahydroajmaline compared with that of ajmaline. It will be of interest to make a reference in this connection to the close relationship established by von Braun and his collaborators between the N-stability of the radicals attached to the basic nitrogen in the codeine series[16] and their action on respiration, and also to allied studies in the conessine series.[17,18] The cyanogen bromide reaction appears to proceed with the two bases on the pattern shown in Chart 1.

With the rupture of the carbinol amine ring in the case of both the diacetyl bases, the acetyl group attached to the ring is apparently eliminated as CH_3COBr, with the formation of a monoacetyl-

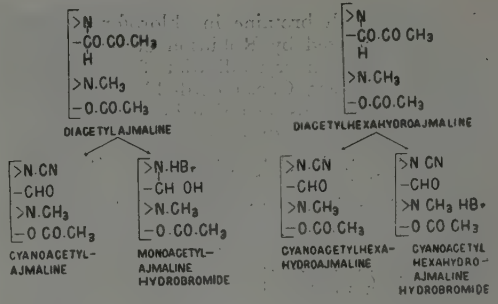


Chart 1

cyano derivative containing a free aldehyde grouping. As to the formation of the hydrobromides, it may be pointed out that a secondary elimination of HBr from the bromo alkyls resulting from the action of BrCN on tertiary amines has been noted by von Braun, and was also encountered with this reaction in the conessine series (loc. cit.). The formation of the monoacetylajmaline hydrobromide would indicate the comparative instability of the acetyl group attached to the carbinol amine ring and it is apparently hydrolysed off under the experimental conditions of this reaction, in which no special care was taken to completely free the reactants from moisture. It is also of interest to note that the reduction of the benzene ring in ajmaline brings about considerable increase in the basic strength of the indole nitrogen, with the result that a stable cyanohexahydroajmaline hydrobromide salt was found in the BrCN reaction with the base. It may be further stated that the use of chloroform employed by von Braun as a medium for the cyanogen bromide reaction (loc. cit.) proved altogether inadequate in the case of the ajmaline base, and that it proceeded smoothly in ethereal solution, as had also been observed with this reaction in the conessine series.

Further work on problems arising out of the present study is in progress with particular refernce to the synthesis of simpler compounds with a carbinol-amine grouping on the general model of the ajmaline molecule.

Experimental

Reduction of Ajmaline and Neoajmaline with Borohydride.—Ajmaline (1 g.) was dissolved in ethyl alcohol, freshly distilled over lime (10 ml.) and an aqueous solution of borohydride (0.5 g. in 10 ml.) was added to it, when the reaction mixture became slightly turbid. More ethyl alcohol was then added drop by drop and shaken

until a clear solution was obtained, and the reaction mixture was allowed to stand overnight at room temperature (25°C.). Any excess of borohydride was decomposed by cautious addition of dilute acetic acid. The solvent was removed on water bath, and the residue rubbed with ammonia and crushed ice. The crystalline precipitate thus obtained was filtered and washed with cold dilute ammonia (yield 0.9 g., m.p. 200-201°C.). Crystallised from methanol (rhombic plates) it melted at 203-205°C. Robinson et al. carried out the reduction in boiling aqueous methanol with potassium borohydride and obtained dihydroajmaline prisms, m.p. 200°C. Neoajmaline treated with borohydride in the same manner gave an identical dihydro product.

Reduction of Isoajmaline with Borohydride.—Isoajmaline was also treated with borohydride as in the case of ajmaline. The dihydro base finally obtained (yield 90%) crystallised from dilute methanol in rectangular plates which melted at 190-92°C. Robinson et al. recorded 188°C. as the melting point of dihydroisoajmaline.

Attempted Isomerisation of Dihydroajmaline.—Dihydroajmaline (0.25 g.) was refluxed in methanolic caustic potash (25 ml., 2% solution w/v) for 5 hours. The solvent was removed on the water bath and the thick viscous residue rubbed with crushed ice. The crystalline product thus obtained was filtered and washed free of alkali with ice-cold water. On re-crystallisation from methanol it melted at 203-205°C. (undepressed on admixture with dihydroajmaline).

On further refluxing for 12 hours with stronger methanolic caustic potash solution also (25 ml., 4% w/v), dihydroajmaline was recovered unchanged.

Borohydride Reduction of Hexahydroajmaline.—Hexahydroajmaline (0.2 g.) was dissolved in warm ethyl alcohol (freshly distilled over lime) and was treated with sodium borohydride (0.2 g.) in the manner already described. The product obtained on basification with ammonia became crystalline on standing. It was filtered, washed and crystallised with dilute methanol (prismatic rods), darkened at 210°C. onwards, shrank at 225°C. and melted at 228-30°C.

Catalytic Hydrogenation of Dihydroajmaline.—Dihydroajmaline (0.25 g.) was taken up in 50% acetic acid (20 ml.) and hydrogenated in the presence of Adams catalyst (0.025 g.). The reduction was complete in one hour. After filtering off the catalyst, the filtrate was concentrated to half the volume *in vacuo*. The precipitate obtained on the addition of ammonia crystallised from dilute methanol in prismatic rods which melted at 230°C. and showed no depression in m.p. on admixture with the product obtained by the borohydride reduction of hexahydroajmaline.

Bromination of Ajmaline.—Ajmaline (0.3 g.) in dry chloroform (4 ml.) was titrated with bromine solution in dry chloroform (1.5% w/v) with ice cooling. Absorption was very rapid and the reaction mixture gave test for free bromine when 11.5 ml. of the bromine solution had been added (4.9 ml. = 1 Br.). The solvent was removed *in vacuo* at room temperature. The micro-crystalline residue obtained on complete removal of the solvent darkened at 210°C. onwards and frothed up at 298-300°C. with decomposition (295°C. recorded by Robinson as the m.p. (decomp.) for anhydrous bromoajmaline). On crystallisation from ethyl alcohol it showed m.p. 235-39°C. with shrinking at 225°C. Siddiqui and Siddiqui recorded 230°C. as the melting point (decomp.) of the bromo derivative. After drying *in vacuo* over P_2O_5 at 100°C., this melting point remained unaffected.

In another experiment ajmaline (0.5 g.) dissolved in chloroform (8 ml.) was treated with bromine in chloroform (1.58 % w/v), drop by drop with ice cooling. After about one atom of bromine had been added, slight turbidity started appearing on further addition which vanished on stirring. When 13.3 ml. had been added (equivalent 1.7 Br) the addition of a further drop of bromine solution gave a persistent turbidity with a tendency to partial crystallisation. Bromine addition was stopped at this stage and the solvent removed *in vacuo*. The white semi-crystalline residue was freed from any unreacted ajmaline by repeated extraction with ethyl acetate and repeatedly crystallised from methanol when it formed stout prismatic hexagonal plates melting at 278-82°C. as against 235-39°C. recorded for the bromo derivative crystallised from methanol in the preceding experiment. The product was fairly soluble in water from which it crystallised in cauliflower-like aggregates of silky needles melting at 230°C. (decomp.), which on crystallisation from methanol reverted to the prismatic form and showed m.p. 268-70°C. (decomp.).

Monobromoajmaline.—Bromoajmaline, m.p. 239°C. (decomp.), dissolved in dry ethyl acetate, was saturated with dry ammonia gas. The precipitated ammonium bromide was filtered off and the solvent removed on the water bath. The solution of the residue in methanol gave on slow evaporation fine colourless needles which melted at 182°C. (decomp.). The melting point was identical with that of the product obtained after Robinson's

method on treatment of the bromination product with aqueous ammonia. (In the case of conessine, it was possible to obtain a dibromo base on treatment of the bromine addition product (dibromoconessine dihydrobromide) with dry ammonia in organic solvents).

Bromination of Isoajmaline.—Isoajmaline (0.206 g.) in dry chloroform was titrated with bromine solution in dry chloroform (1.58% w/v) with ice cooling. The reaction mixture showed the presence of free bromine (tested with starch-potassium iodide paper) when 0.13 g. had been added (0.11 g. = 1 mole). The solvent was removed under reduced pressure at room temperature (25 °C.). The residue crystallised on rubbing with methanol in triangular plates, m.p. 225-228 °C. (decomp.). (Robinson et. al. recorded 288-289 °C. (decomp.) as the melting point for 'anhydrous' bromoisoajmaline).

Action of Bromine on Dihydroajmaline.—A well-cooled solution of dihydroajmaline (0.24 g.) in dry chloroform was treated with bromine in dry chloroform (1.58% w/v). The absorption of bromine appeared to be very slow as judged by the disappearance of the yellow colour. One mole of bromine was however added and the mixture kept in the cold for about an hour. The solvent was then removed *in vacuo* at room temperature (25 °C.) and the residue which became powdery on rubbing with ether was filtered and washed well with ether. The amorphous powder decomposed with charring at 220-222 °C. It was sparingly soluble in water and the aqueous solution produced a precipitate with aqueous silver nitrate. The amorphous powder, which failed to crystallise from methanol or dilute methanol, was treated with ammonia in aqueous medium, and the liberated base crystallised from methanol, when it melted at 200-201 °C. showing no depression in melting point on admixture with dihydroajmaline.

Bromination of Methylajmaline.—Methylajmaline (0.33 g.) in dry chloroform was titrated with bromine in dry chloroform (1.38% w/v) with ice cooling. The reaction mixture showed the presence of free bromine when 16.5 ml. (ca. 3 Br) had been added. The supernatant liquid was decanted from the precipitate which formed with the addition of bromine. On rubbing with ether the yellowish sticky residue turned into a nearly colourless powder, which when washed well with ether darkened from 180 °C. onwards, and melted with decomp. at 290 °C. The bromo product was then treated in dry ethyl acetate suspension with dry ammonia gas, the resulting solution of the liberated base filtered off from ammonium bromide and the filtrate freed of the solvent *in vacuo*. The residue on complete removal of the solvent melted at 120 °C. but failed to crystallise. It was characterised as methylisoajmaline through its hydriodide, which on crystallisation from methanol melted at 198 °C. (decomp.). (Robinson et al. have recorded m.p. 195-96 °C.).

Reaction of Bromine on Hexahydroajmaline.—Hexahydroajmaline (0.3 g.) was dissolved in an excess of chloroform (about 30 ml.) in which it is difficultly soluble. The solution was well cooled in ice and titrated with bromine solution (1.38% w/v). Absorption was rapid and the reaction mixture gave the test for free bromine when 6 ml. of the solution had been added (about 1 Br).

The residue obtained on complete removal of the solvent *in vacuo* at ordinary temperature was found to be readily soluble in water and gave a precipitate with silver nitrate. The base liberated from the aqueous solution of the product with dilute alkali and crystallised from ethyl alcohol (square plates) showed m.p. 150 °C., as recorded for hexahydroajmaline by Robinson et al.

Action of Bromine on Diacetylhexahydroajmaline.—Diacetylhexahydroajmaline on treatment with bromine under conditions already described gave a water-soluble reaction product from which the base was recovered unchanged. It was characterised through its perchlorate m.p. 190-192 °C., undepressed on admixture with an authentic sample of the salt.

Action of Cyanogen Bromide on Diacetylajmaline.—Diacetylajmaline (5 g.) was dissolved in ether (50 ml.) with the help of a little ethyl acetate, and the solution concentrated to about 120 ml. To the solution was added drop by drop a solution of 1.5 g. cyanogen bromide (1.15 mole) in ether (15 ml.) with efficient ice cooling and stirring. The gelatinous precipitate, which separated out during the addition in the course of about 10 minutes, turned into a granular powder on rubbing. After keeping in the cold for about 3 hours the ethereal solution was filtered, repeatedly washed with small quantities of water, dried over sodium sulphate and allowed to concentrate by slow evaporation overnight. The product thus obtained (5.0 g.) crystallised from ethyl acetate in colourless, stout, prismatic rods and hexagonal plates which were soluble in hot ethyl acetate, very sparingly so in ether and alcohol, insoluble in water, melted at 205-207 °C. and analysed for monoacetylcyanoajmaline.

Calculated for $C_{23}H_{27}O_3N_3$: C, 70.23; H, 6.87; N, 10.88. Found on drying at 100 °C. *in vacuo*: C, 69.57; H, 6.96; N, 10.75.

The granular precipitate obtained in the reaction (0.8 g.) melted at 250-252 °C. and when repeatedly crystallised from a large quantity of ethyl acetate helped with a little ethyl alcohol finally gave colourless crystals which showed m.p. of 266 °C., were soluble in water, very sparingly so in ethyl acetate, almost insoluble in ether or petroleum ether and analysed for monoacetylajmaline hydrobromide.

Caculated for $C_{22}H_{28}O_3N_2.HBr$: C, 58.79; H, 6.68; N. 6.46; Br, 17.81. Found on drying at 100 °C. *in vacuo*: C, 58.61; H, 6.11; N. 6.10; Br, 17.99.

The equeous solution of the hydrobromide was basified with ammonia and the liberated base on recrystallisation from dilute alcohol melted at 212-214°C. and analysed for monoacetylajmaline.

Calculated for $C_{22}H_{28}O_3N_2$: C, 71.73; H, 7.60; N, 7.60. Found on drying at 100°C. *in vacuo*: C, 71.77; H, 7.87; N, 7.52.

In the process of working up the reaction mixture small quantities of crystalline products, probably resulting from side reactions and melting at 194-196 °C. and 220-224 °C., were also obtained but were not followed up.

Hydrolysis of Monoacetylcyanoajmaline—Isoajmaline.—Monoacetylcyanoajmaline (0.5 g.) was refluxed over water bath with methanolic potassium hydroxide (5% w/v, 10 ml.). The hydrolysis proceeded at a very slow rate with the formation of sodium carbonate and evolution of ammonia. After about 50 hours, the reaction mixture gave on keeping in the cold for 48 hours, a bulky crop of colourless crystals which was filtered off from the supernatant liquid, washed with a little ice-cold methyl alcohol and then with water (yield, 0.18 g.). On crystallisation from methyl alcohol it gave colourless stout rods which melted at 256 °C. and analysed for isoajmaline (m. p. 265 °C.). On further refluxing, the mother liquor from the reaction crystallisate gave, after about another 40 hours, 0.17 g. of the hydrolysed base (total yield, 0.35 g.).

Action of Cyanogen Bromide on Diacetylhexahydroajmaline. — Diacetylhexahydroajmaline (0.57 g.), obtained by treating the crystalline perchlorate of the base with ethereal ammonia in ether suspension, was dissolved in ether (3 ml.). To the ethereal solution of the base was added drop by drop, a solution of cyanogen bromide 0.2 g. (a little in excess of one mole) in dry ether (5 ml.) with efficient ice cooling and stirring. No immediate precipitation followed, as had happened in the case of this reaction with diacetylajmaline. The reaction mixture was then allowed to stand in the cold for about 24 hours, in the course of which a thick jelly-like mass separated out which was repeatedly triturated with ether. The combined ethereal extract was freed of the solvent on the water bath and the residue thus obtained was crystallised from methanol, out of which it came out in colourless needles which were readily soluble in ethyl alcohol and ethyl acetate, sparingly soluble in ether, less so in petroleum ether, melted at 146-148 °C. and analysed for monoacetylcyanohexahydroajmaline.

Calculated for $C_{23}H_{33}O_3N_3$: C, 69.17; H, 8.27; N, 10.52. Found on drying at 100 °C. *in vacuo*: C, 69.39; H, 8.40; N, 10.39.

The ether insoluble, gelatinous residue was completely freed of the solvent, taken up in water and extracted with small quantities of ethyl acetate. The colourless residue left on removal of the solvent from the aqueous layer on the water bath, crystallised from a mixture of absolute alcohol and ethyl acetate in colourless needles (m.p. 256-257 °C.) which were freely soluble in water and alcohol, almost insoluble in ethyl acetate and ether, and analysed for monoacetylcyanohexahydroajmaline hydrobromide.

Calculated for $C_{23}H_{33}O_3N_3.HBr.H_2O$: C, 55.42; H, 7.22; N, 8.42; Br, 16.06. Found on drying at 100 °C. *in vucuo*: C, 55.82; H, 7.03; N, 8.31; Br, 16.21.

The free base obtained from the hydrobromide salt on liberation with ammonia crystallised from methanol in colourless needles which melted at 146-148 °C. and showed no depression in the melting point on admixture with the cyano derivative obtained directly from the ether soluble portion of the reaction product.

The total yield of the cyano base was nearly theoretical, and derived in about equal proportions from the ether soluble and the ether insoluble fractions of the cyanogen bromide reaction product.

Acetylation of Ajmaline and Hexahydroajmaline.—*Diacetylajmaline and Diacetylhexahydroajmaline.*—In the preparation of diacetylajmaline needed for the BrCN reaction, considerable difficulty was experienced in following the method employed by Robinson et al. (refluxing with acetic anhydride in benzene solution for 12 hours). Apart from being time consuming and complicated in its working it was noted that slight impurities in the reactants or the solvent very adversely affected the yield, and a simpler method

was, therefore, worked out for the acetylation of these bases which consisted in heating the components directly for 5 minutes in the water bath, removing acetic anhydride completely *in vocuo* and crystallising the product when diacetylajmaline (hexagonal prismatic rods, m.p. 192-194°C.) and diacetylhexahydroajmaline (isolated as perchlorate needles, m.p. 190-192°C.) were obtained in about 70% yield.

Summary

The action of bromine on ajmaline and its various derivatives has been studied in detail to elucidate the mechanism of this reaction, and earlier points of disagreement in respect of the chemical characteristics of ajmaline have been experimentally checked up for clarification. Further, on the basis of studies in the action of cyanogen bromide on the diacetyl derivatives of ajmaline and hexahydroajmaline, the antifibrillant cardiac action of ajmaline has been co-related with the N-stability of the carbinol-amine structure, which appears to function as a 'cardiophore' grouping in the ajmaline molecule.

Acknowledgement

The auothors wish to acknowledge with thanks the kind courtesy of Messrs Boehringers of Mannheim in supplying ajmaline needed for this work. Their thanks are also due to Mr. Sardar Ali Khan for his assistance in the preparative work and to Dr. M. Ikhlas Khan for testing the various ajmaline derivatives for their cardiac action.

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PHARMACOLOGICAL INVESTIGATION OF SERPAJMALINE, AN ALKALOIDAL FACTOR OF RAUWOLFIA SERPENTINA

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Recently S. Siddiqui[1] reported the discovery of a pharmacologically active *Rauwolfia* alkaloidal fraction which was named 'serpajmaline'. This fraction includes the alkaloids serpentine, serpentinine and ajmaline, two unknown substances and a carbohydrate-like substance, and possesses remarkable blood pressure reducing properties without any sedative action, as recently described by us.[2] It has long been known that the *Rauwolfia Serpentina* alkaloids, reserpine[3] and rescinnamine,[4] have a greater blood pressure reducing action than the other alkaloids but it has not hitherto been possible to answer the question whether the hypotensive action is primarily inherent or whether it depends only on its central depressive action. It thus seems noteworthy that serpajmaline, which contains no reserpine, has a strong blood pressure reducing action without central side effects. In the present work we have therefore extended our previous results on the blood pressure reducing action of serpajmaline and have investigated more closely various peripheral effects of this fraction.

Methods

Blood Pressure Experiments on Dogs.—Using a *bloodless method*, we prepared the art. carotid externally looped in a skin sheath, following Wezler and Thauer.[5] The blood pressure was measured as by Riva Rocci on the non-anaesthetised animal.

For the *sanguinous method* we used a Hg manometer for recording the arterial pressure from the carotid. To prevent coagulation we injected 500 I.U./kg. Vetren with a preliminary 500 I.U. in the supply tube. Heart frequency was recorded with the ordinate recorder developed by Heuwing. Narcosis; Pentothal 15 mg./kg. and phenobarbitone 25 mg./kg. intravenous, phenobarbitone 75 mg./kg. intraperitoneal. Injections in the V. Femoralis.

Blood Pressure Experiments on Cats.—Recording of the arterial pressure from the carotid with a Hg manometer. As regards preventing coagulation see dogs. Injection in the Femoralis. Narcosis: Chloralose 80 mg./kg. intraperitoneal. Injections in the V. Femoralis.

Intestine and Spleen in situ.—With cats under the above Chloralose narcosis or with spinal cats, a bubble sonde was introduced into the jejunum through the oesophagus and the stomach, and this was connected to a water manometer. The recording of the spleen volume was made pethysmographically and connected to a piston recorder.

Isolated organs.—Terminal ileum of guinea-pigs was used in the usual arrangement following Magnus.

Temperature Monitoring.—For recording the body temperature of rabbits we used Hartmann and Braun's apparatus.

Preparation.—The serpajmaline was isolated by Dr. S. Siddiqui in the Chemical Research Division of the Central Laboratories.

Experiments

(1) **Action of Serpajmaline on the Circulation.**—Serpajmaline has a blood pressure reducing action on dogs and cats, dogs being more sensitive than cats. The magnitude of the blood pressure reduction depends on the condition of the experimental animal, the depth of narcosis, and the dosage.

(*a*) *Effect on the Blood Pressure of Anaesthetised Dogs*

Small doses of serpajmaline, 0.25-1 mg./kg., injected intravenously, produce a transient fall of blood pressure of a few mm. Hg. After 2 mg./kg., however, the blood pressure was reduced by 40 mm. Hg for about 20 min. on the average. Figure 1 shows the effect on the blood pressure of repeated injections of serpajmaline at intervals of 10 min. From this figure it can be seen that serpajmaline is still capable of depressing the blood pressure even after it has been greatly reduced by repeated injections.

Reserpine has no effect after rapeated injections. After a reduction of the blood pressure has been produced once, reserpine is incapable of depressing the blood pressure even once more. The bradycardiac action of serpajmaline can be

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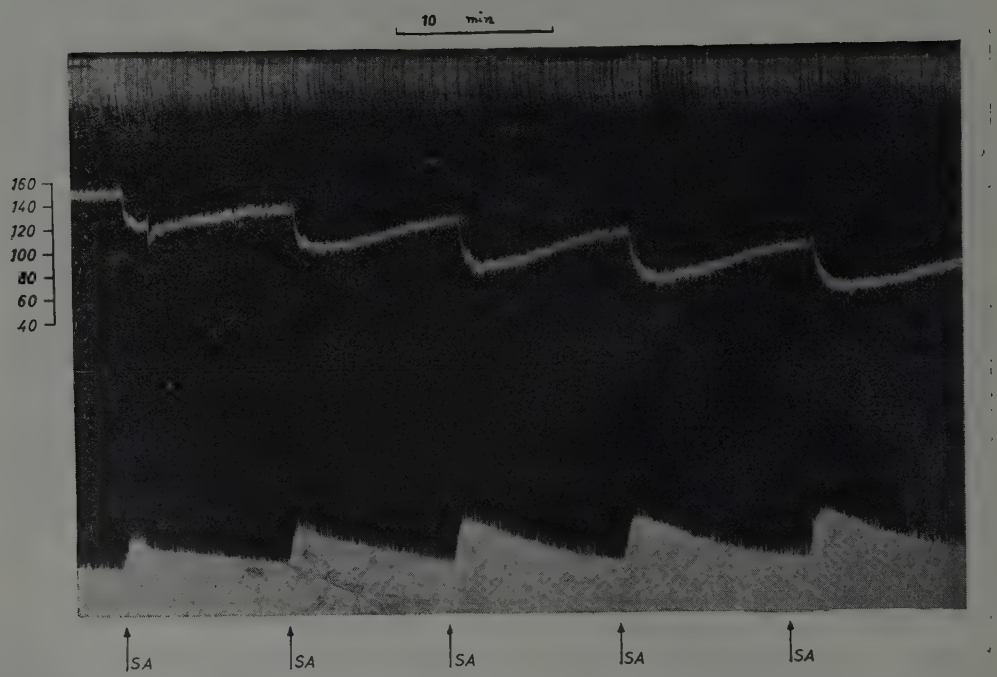

Fig. 1.—The effect on the blood pressure and heart frequency after repeated injections of serpajmaline. Dog 25 kg., Pentothal-phenobarbitone anaesthesia. Recording of blood pressure with Hg manometer from the carotid. Above: respiration; middle: blood pressure; and below: heart frequency. Time in minutes. After repeated injections of serpajmaline, 2 mg./kg. i.v., at ten-minute intervals, the blood pressure falls in steps. The bradycardia increases.

seen clearly from Fig. 1; this action is also cumulative.

At this dosage (2 mg./kg.) reserpine has no acute blood pressure reducing action. A transient blood pressure reduction of short duration is obtained in the control test (0.5% acid acetic) (see Fig. 2). The hypotensive action of reserpine does not show until 1-2 hours after the injection, and the magnitude of the reduction is 20-40 mm. Hg. Because of these circumstances, a quantitative comparison is very difficult, if not impossible. It may however be stated that, in comparison with reserpine, serpajmaline has a much stronger acute depressive action on the blood pressure (see Fig. 2).

(*b*) *Effect on the Conscious Dog with Carotid in External Skin Sheath*

In the experiments on the conscious dog with carotid in external skin sheath there was general concordance with the results on anaesthetised animals. The effect of serpajmaline was stronger

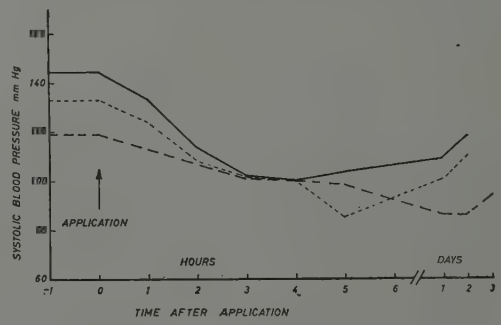

Fig. 3.—The effect of single applications of serpajmaline or reserpine on systolic blood pressure in conscious dogs. Dogs with carotid in external skin sheath.

————: Serpajmaline 5 mg./kg. intramuscularly. Average values of 3 dogs.

.....: Serpajmaline 5 mg./kg. orally. Average values of 6 dogs.

- - - - - - -: Reserpine 1 mg./kg. intramuscularly. Avreage values of 3 dogs.

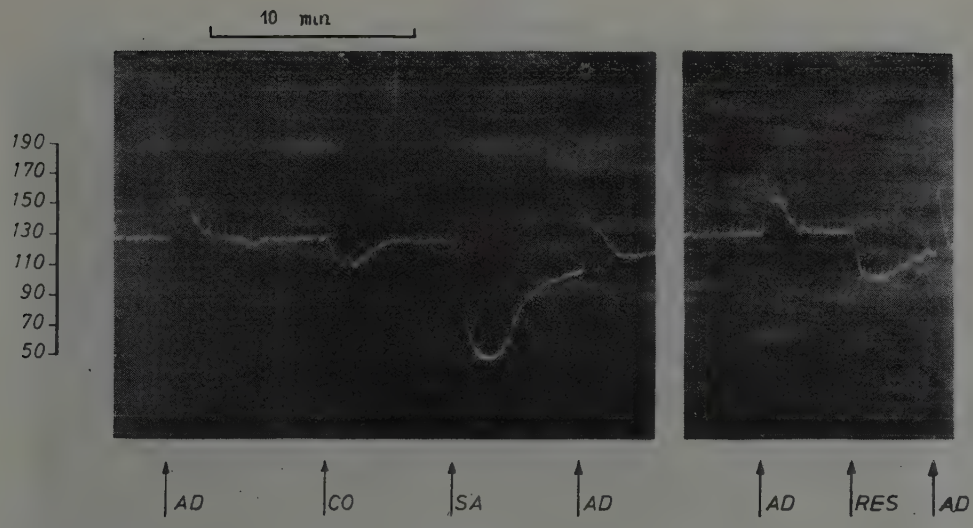

Fig. 2.—Comparison of the acute blood pressure effect of serpajmaline and reserpine. Dog 15 kg., pentothal-phenobarbitone anaesthesia. Recording of blood pressure with Hg manometer from the carotid. Above: respiration; below: blood pressure. Time in minutes. The acute fall of blood pressure after 1 mg./kg. reserpine (RES) corresponds to the control (CO) whereas serpajmaline (SA) 1 mg./kg. depresses the blood pressure clearly and reduces the effect of adrenalin (AD) 12γ.

and more enduring on conscious animals than on anaesthetised ones. In all cases, 5 mg./kg., was given orally and intramuscularly. This dose resulted in a reduction of 40-60 mm. Hg in the blood pressure. After oral application of 5 mg./kg., serpajmaline also has a definite long-term action, up to 24 hours. During the period of hypotension there was no miosis, no relaxation of the nictitating membrane, and no diarrhoea or sedative action. In comparison with serpajmaline, the effect of reserpine, 1 mg./kg. intramuscular, can be seen in Fig. 3. Orthostatic callapse did not occur after serpajmaline when the dog jumped from the table, and a rise in blood pressure could still be obtained when the dog was given exercise (Fig. 4).

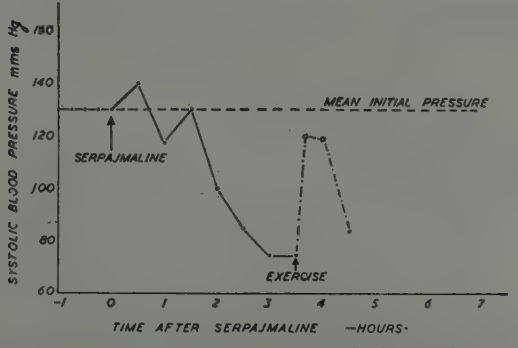

Fig. 4.—The effect of exercise on the hypotension produced in a conscious dog by a single oral dose of serpajmaline. (Dose 5 mg./kg.). Dog 20 kg., with carotid in external skin sheath.

(c) Effect of Serpajmaline on the Blood Pressure and Spleen Volume of Anaesthetised and Decapitated Cats

The effect of serpajmaline on the blood pressure of anaesthetised cats is weaker than on dogs. Five mg./kg. serpajmaline injected intravenously into the anaesthetised cat had little effect on blood pressure. On the spinal cat there is sometimes a short-term rise of blood pressure. Rothlin[6] has described a rise of blood pressure with various ergot alkaloids. However, notwithstanding the rise in blood pressure, there is an increase in the spleen volume of the spinal cat (Fig. 5).

(2) Adrenergically Inhibiting Effects of Serpajmaline.—In anaesthetised and decapitated cats, serpajmaline inhibits the pressor action of adrenalin and noradrenalin. Similarly, the contraction of the nictitating membrane after adrenalin is inhibited (Figs. 5,6). On the other hand, the contraction of the nictitating membrane after pre-ganglionic stimulation is inhibited to only a small degree (Fig. 6). The block which is to be seen in Fig. 6 after peripheral vagus stimulation is discussed elsewhere. The contraction of the spleen volume after adrenalin is inhibited by serpajmaline (Fig. 5).

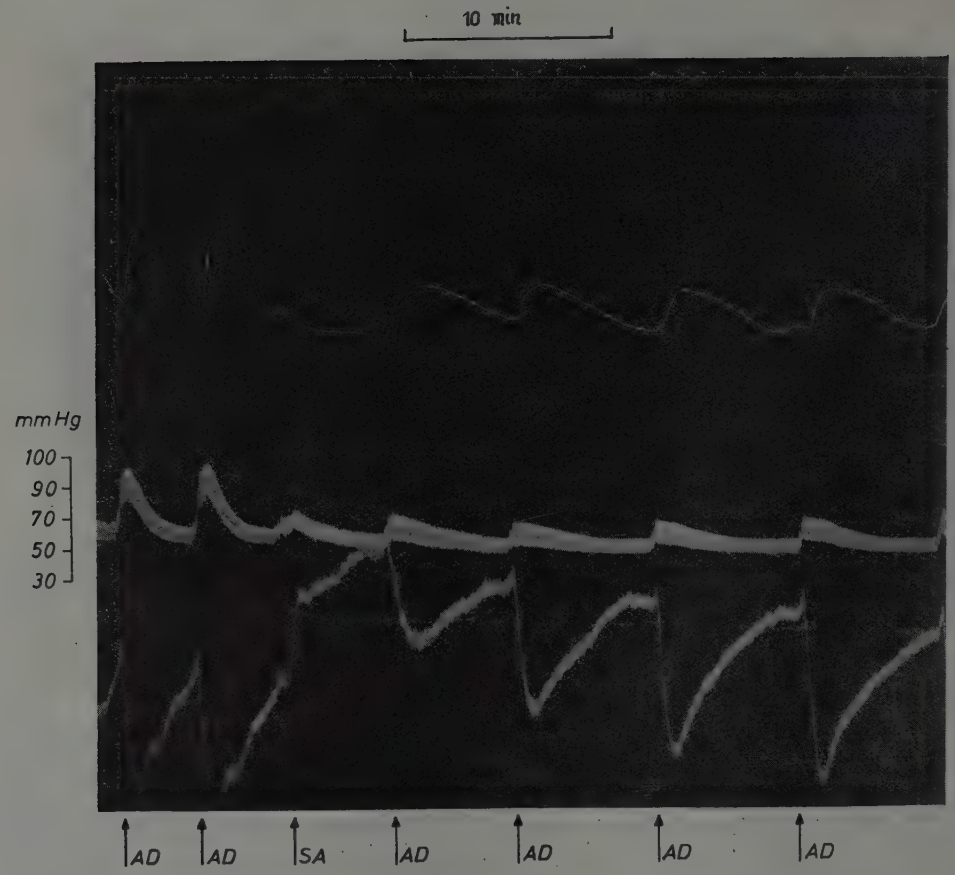

Fig. 5.—Inhibition of the increase of blood pressure caused by adrenalin, contractions of membrana nictitans and of spleen valume. Spinal cat, 2.2 kg., artificial respiration. Recording of blood pressure with Hg manometer from the carotid. Intravenous injections of adrenalin (AD) 10γ after serpajmaline 10 mg./kg. i.v. (SA). Above: membrana nictitans; middle: blood pressure; and below: spleen volume. Time in minutes. Transient rise of blood pressure and increase of spleen volume after serpajmaline. The adrenalin effects are inhibited.

The tachycardiac effect of adrenalin and noradrenalin on anaesthetised dogs is definitely reduced, the inhibiting effect for noradrenalin being greater than for adrenalin, as can be seen from Table 1.

TABLE 1

Frequency rise after	Adrenalin 30%	Nor-adrenalin 26%
After serpajmaline 2 mg./kg. i.v.	19%	9·5%

The inhibitory action of adrenalin on the intestine is slightly weakened (Fig. 7).

(3) **Effect of Serpajmaline on Body Temperature.**—No variation of the body temperature of rabbits was observed after subcutaneous injection of 1-5 mg. /kg. serpajmaline.

(4) **Action of Serpajmaline on the Intestine.**—It has long been known that some *Rauwolfia* alkaloids have an exciting effect on the stomach intestinal tract.[7] With serpajmaline also, after injection of 10 mg./kg. i.v. in cats there is an increase of motility and tone in the intestine

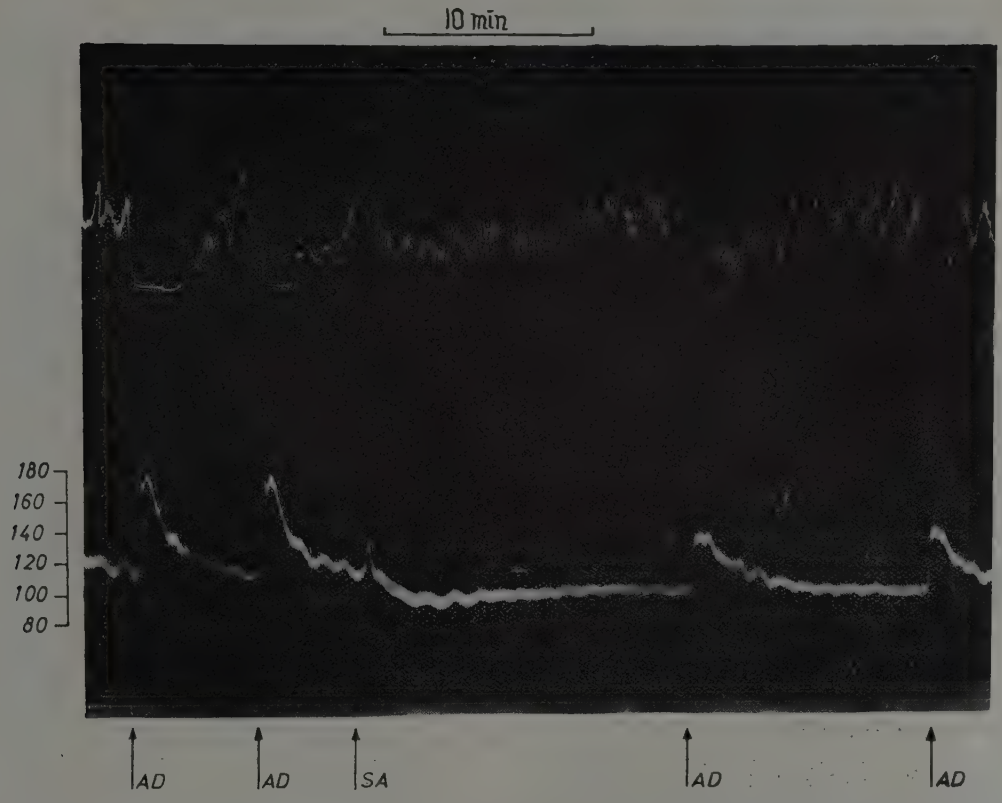

Fig. 7.—Influence of serpajmaline on the contractions of the jejunum in situ. Cat 13 kg. Chloralose anaesthesia. Recording of blood pressure with Hg manometer from the carotid. Above: volume of jejunum; and below: blood pressure. Time in minutes. After serpajmaline 10 mg./kg. (SA) the pressor effect of adrenalin (AD) on the blood pressure is reduced. The inhibitory effect of adrenalin on the intestine is slightly reduced. The intestine reacts with enhanced tone and contractions.

in situ. On the isolated ileum of guinea-pigs serpajmaline in concentrations of 1×10^{-5} inhibits contractions induced by barium chloride and acetylcholin.

Discussion

The hypotensive action of serpajmaline is not complicated by any of the side effects encountered with compounds such as reserpine or rescinnamine, the ganglion blockers or the anti-adrenalin drugs. Thus there is no sedative action, anorexia or diarrhoea or postural hypotension. Serpajmaline induces a bradycardia of the sinus. On the basis of the anti-adrenergic activity it is to be assumed that the inhibition of the adrenergic innervation of the vessels is the cause of the effect on the circulation. However, this inhibition is certainly not necessarily or exclusively the sole cause. The action of the serpajmaline on the relieving reaction of the carotid sinus[2] provides evidence that not only the periphery of the vessels but also central mechanisms of the circulation are affected by the action. But it is difficult to draw conclusions as to central activity of a drug known to possess anti-adrenalin activity. Spinal animals show a transient rise of blood pressure after serpajmaline. Dihydro ergot alkaloids raise the blood pressure in spinal animals and lead to reductions of blood pressure only when there is an intact connection between medulla and the upper thoracic marrow,[8] which points to medullary centres being involved. The fact that serpajmaline is a mixture of five bases

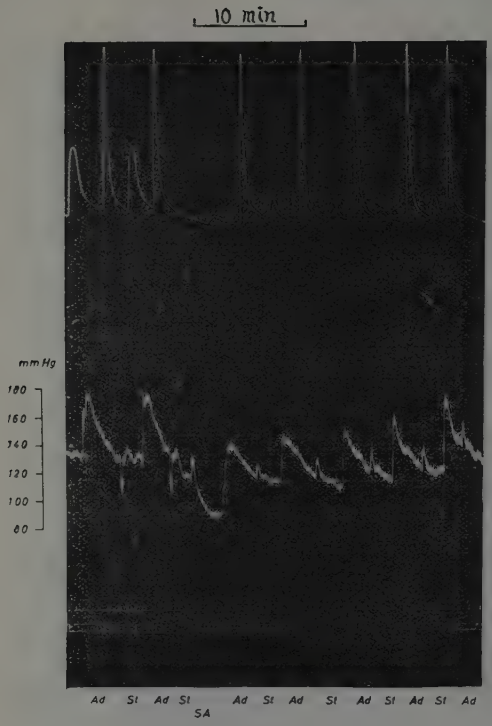

Fig. 6.—Influence of serpajmaline (*a*) on the effect of adrenalin on the membrana nictitans and the blood pressure, (*b*) on the contractions of the nictitans and the blood pressure after supramaximal stimulation of the vagus-sympathicus. Cat 2.3 kg. Chloralose anaesthesia. Recording of blood pressure with Hg manometer from the carotid. Above: membrana nictitans; and below: blood pressure. Intravenous injections of adrenalin (AD) 10γ, stimulation of the vago-sympathicus (ST). After serpajmaline 8 mg./kg., lowering of the blood pressure and inhibition of the contractions of the membrana nictitans and pressor effect produced by adrenalin. Blockage of the peripheral vagus effect. No effect on the nictitans membrane after preganglionary stimulation.

and carbohydrates renders more difficult the pharmacological interpretation of the complex mechanism producing the action. Further experiments for elucidating the pharmacology and toxicology of serpajamaline will be treated in greater detail in a separate paper.

Summary

Serpajmaline (5 mg./kg.) reduces the systolic blood pressure of conscious dogs for at least 24 hours. The drug is effective by both the oral and the intramuscular routes.

In anaesthetised animals, serpajmaline reduces blood pressure and has a bradycardiac action. The nictitating contraction, the pressor, and the tachycardiac actions of adrenalin and noradrenalin are inhibited. In spinal animals, transient rises of blood pressure are observed, together with an increase of spleen volume. Serpajmaline increases motility and tone of the intestine in situ. In vitro, serpajmaline inhibits the action of barium chloride and acetylcholine on the intestine.

Acknowledgement

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THE PHARMACOLOGY AND TOXICOLOGY OF SERPAJMALINE

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Serpajmaline is a pale brown powder, readily soluble in water, obtained from the fresh roots of *Rauwolfia serpentina*;[1,2] it contains the alkaloids serpentine, serpentinine and ajmaline, as well as two unidentified substances and some carbohydrate-like material. Serpajmaline contains neither reserpine nor rescinnamine.

Deininger[3] claimed that serpajmaline was a more potent hypotensive drug than reserpine and, further, that it did not have the latter's central sedative effects.

Deininger[4] later investigated the action of serpajmaline on anaesthetised cats and on conscious and anaesthetised dogs and studied its effects on the ileum, both isolated and in situ.

In this paper we record the results of further pharmacological and toxicological experiments on serpajmaline.

Methods

Studies in Conscious Animals

1. *Acute Toxicity*

Mice.—The acute toxicity of serpajmaline was determined by the intravenous, intraperitoneal and oral routes on female fawn mice (GFF strain), and by the oral route in female albino mice (A2G strain). The mice weighed between 16 and 22 g. Serpajmaline solutions were freshly prepared in distilled water immediately before use, and each dose was administered to a group of not less than five mice. Percentage mortalities were assessed seven days after administration, and the LD 50 values were determined by the method of de Beer.[5]

Rats.—The acute toxicity of serpajmaline was determined by the intravenous, intraperitoneal and oral routes on male rats (WAG strain, 100 to 150 g.).

The drug was administered to groups of five rats, and the percentage mortalities were assessed seven days later.

2. *Subacute Toxicity*

The subacute toxicity of serpajmaline was investigated in albino mice (A2G strain).

Groups of five male and five female mice received daily oral doses of serpajmaline for twelve weeks. The dose was 125 mg./kg., which is approximately one-fifth the oral LD 50 value for this strain. Each mouse was weighed daily; the oxygen consumption rate of each group was measured daily during the third week of treatment only. Two control groups, each of five mice, were included in this test. After twelve weeks' treatment the group oxygen consumption were measured on two consecutive days and the mice were then paired for breeding. They were not dosed with serpajmaline during the breeding period.

3. *Effect on Barbiturate Activity in Mice*

One hundred and fifty male mice (GFF strain, 16 to 26 g.) were injected intraperitoneally with serpajmaline (50 mg./kg. one-fifth LD 50). At intervals ranging from one hour to seven days after receiving serpajmaline, the mice were injected intraperitoneally with hexobarbitone (100 mg./kg.,5 mice per group) or intravenously with barbitone (275 mg./kg., 10 mice per group). The sleep-times of the mice receiving hexobarbitone were measured in a cabinet thermostatically controlled at 37 °C.; the induction periods of the barbitone-injected mice were measured at room temperature.

Control groups, receiving barbiturates only, were included with each experiment to allow for day-to-day variations in barbiturate response.

4. *Analgesic Activity in Mice*

Serpajmaline was tested for analgesic activity in mice by the method of Woolfe and Macdonald.[6] Two groups of ten male mice (GFF strain, 17 to 22 g.) were injected intraperitoneally with serpajmaline (10 or 100 mg./kg.). At intervals after the injection, ranging between 10 minutes and 3 hours, the mice were placed individually on a hot plate maintained at 55 °C. and their reaction times were recorded. The reaction time is defined as the intervals between placing the mouse on the

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hot plate and observing signs that indicate the onset of pain, e.g., kicking, paw retraction.

5. Effect on the Blood Pressure of Renal Hypertensive Rats

The rats used in these experiments were made hypertensive by a modification of the renal clamping technique first described by Goldblatt.[7] Young rats of both sexes (40 to 100 g.) were anaesthetised with ether, and a small silver clamp was placed on one renal artery. This reduced but did not completely prevent the flow of blood to the kidney. On recovering from the anaesthetic the rats were maintained in an environment at 28° C. and given free access to a commercial rat cube diet[8] and tap water. Two to three months later a stable chronic renal hypertension was apparent in about 70% of the rats; those that did not become hypertensive (i.e. their blood pressure values were < 140 mm. Hg.) were rejected.

The blood pressures of the hypertensive rats were determined at regular intervals by the tail plethysmographic method of Byrom and Wilson.[9] Blood pressures were assessed under light ether anaesthesia; because of this the determinations were made at intervals of not less than three hours.

Five groups of ten renal hypertensive rats were used in this experiment. One group served as controls, and the other four groups were injected intraperitoneally with serpajmaline on four consecutive days. Group 2 received individual doses of 6.25 mg./kg., and groups 3, 4 and 5, 12.5, 25 50 mg./kg., respectively. Blood pressure recordings were made one hour after each injection and 24 hours and 4 days after the fourth dose.

6. Brain Serotonin-Releasing Activity

Nine adult rabbits were used in this experiment. Three served as controls, three received serpajmaline (10 mg./kg.) and three reserpine (1 mg./kg.). All injections were made into the marginal ear veins. Twenty-four hours later the rabbits were killed and their brains were removed, weighed and assayed spectrofluorometrically for total serotonin, by the method of Bogdanski et al.[10]

7. Effect on Blood Sugar Concentration

Three adult rabbits were injected intravenously with serpajmaline (10 mg./kg.); blood samples were taken before, and 5, 15, 30 and 60 minutes after the injection. Blood sugar levels were estimated by the method of Hagedorn and Jensen.

8. Effect on Clotting Time

Three adult rabbits were injected intravenously with serpajmaline (10 mg./kg.). The rabbits were bled before injection and at intervals thereafter, the samples being collected in waxed stoppered tubes. Clotting times were determined to the nearest half-minute, by inverting the tubes once every 30 seconds.

9. Neuromuscular Blocking Activity in Chicks

Serpajmaline was tested for neuromuscular-blocking activity in conscious chicks by the method of Buttle and Zaimis.[11] All injections were given intravenously.

10. Reserpine-like Action in Kittens

One male and one female kitten (900 to 1000 g.) received single oral doses of serpajmaline (50 mg./kg.). The powder was administered in gelatine capsules. The kittens were observed for 6 hours for evidence of parasympathomimetic activity typical of reserpine.

11. Tranquillising Effect in Monkeys

Two female cynomolgus monkeys (3 to 4 kg.) were given serpajmaline, one animal receiving 5 mg./kg., intravenously and the other 50 mg./kg. orally. Two other monkeys in the same weight range received reserpine (1 mg./kg.), either intravenously or orally.

Studies on Anaesthetised Animals

1. Cats

Cats weighing between 2 and 5 kg. were anaesthetised with chloralose (80 mg./kg. intraperitoneal route); blood pressure, respiration and E.C.G. were recorded. The vagus and cervical sympathetic nerves were separated and the responses of the cardiovascular system to peripheral vagal stimulation and of the nictitating membrane to peripheral sympathetic stimulation were recorded. A cannula was inserted into the saphenous vein and injections of saline, adrenaline, noradrenaline, acetylcholine and histamine were administered intravenously.

In two experiments serpajmaline was administered into the lateral cerebral ventricle via a Feldberg cannula,[12] and in another experiment the effects of serpajmaline on the motility of the small intestine in situ were recorded by the method of Straub.

Serpajmaline was administered by intravenous injection, intravenous infusion, intraventricular injection, intraduodenal injection and via an oesophageal catheter directly into the stomach. In several experiments serpajmaline was also injected through a catheter passing down the external jugular vein with its tip terminating at the point where the great veins enter the right auricle.

2. Dogs

Mongrel bitches weighing between 8 and 12 kg. were anaesthetised intravenously or intraperitoneally with chloralose (100 mg./kg.). When necessary anaesthesia was maintained with small doses of sodium pentobarbitone.

The blood pressure, respiration and E.C.G. were recorded, and the drugs were injected either through a cannula in the femoral vein or directly into the duodenum. The responses of the cardiovascular system to peripheral vagal stimulation and of the nictitating membrane to pre-ganglionic stimulation were recorded.

3. Monkeys

Serpajmaline and reserpine were compared in six female cynomolgus monkeys weighing between 3 and 4 kg. The animals were anaesthetised with sodium pentobarbitone (30 mg./kg. intravenously), and the blood pressure, respiration and E.C.G. were recorded. All injections were given through a cannular inserted in the femoral vein.

4. Rabbits

Rabbits were anaesthetised with urethane (1 g./kg.) injected intraperitoneally, and the blood pressure and respiration were recorded. All drugs were injected into the femoral vein.

STUDIES ON ISOLATED TISSUES

In vitro effects of serpajmaline were studied on guinea-pig ileum, rabbit duodenum, non-oestrus rat uterus, chick semispinalis cervicis muscle, perfused rabbit heart and beating rabbit auricles.

1. Guinea-pig Ileum

The ileum was suspended in a 20 ml. bath of oxygenated Tyrode solution at 31°C. Longitudinal contractions were recorded with a frontal writing lever on a smoked drum.

2. Rabbit Duodenum

The duodenum was removed from a freshly killed rabbit and suspended in oxygenated Tyrode solution at 35°C.

3. Rat Uterus

One horn of the uterus of a non-oestrus rat was suspended in a bath of de Jalon solution at a temperature of 31°C. The solution was oxygenated with 95% oxygen and 5% carbon dioxide.

4. Chick Semispinalis Muscle

One of the semispinalis cervicis muscles of the chick was removed under ether anaesthesia and suspended in a bath of oxygenated Tyrode solution at 40.5°C. Longitudinal contracture of the muscle was recorded with a frontal writing lever.

5. Perfused Beating Rabbit Heart

Rabbit hearts were perfused by a modification of the Langendorff technique. The hearts were perfused with McEwen solution[13] at 37°C. and at a perfusion pressure of 40 cms. of water. The perfusion fluid when fully saturated with a mixture of 95% oxygen and 5% carbon dioxide had a pH of 7.4. Coronary flow was recorded with an inflow recorder, and the amplitude was recorded on a smoked drum. Drugs were added to the perfusion fluid via a polythene cannula opening near the coronary arteries so that dilution was reduced to a minimum.

6. Perfused Fibrillating Rabbit Heart

Fibrillation was induced in perfused beating rabbit hearts by electrically stimulating the ventricle and reducing the concentration of potassium in the McEwen solution to three-quarters of the normal amount. After fibrillating for 15 minutes a perfused heart never spontaneously reverted to normal rhythm; further, an injection of sodium chloride, potassium chloride or distilled water from pH 5 to pH 8 did not affect the fibrillation.

7. Rabbit Auricles

The effect of drugs on the maximum drive rate of the rabbit auricles was determined by the method of Dawes[14] as modified by Alles and Ellis.[15]

The heart was removed from a freshly killed rabbit and the auricles were dissected free from other heart muscle and suspended in a bath of oxygenated (95% oxygen and 5% carbon dioxide) Locke solution at a temperature of 29°C. The auricles were stimulated electrically with square wave pulses of 3 m.secs width and an intensity of

Table 1.—The Acute Toxicities of Serpajmaline in Mice and Rats.

Species	Strain	Sex	LD 50 value — mg./kg.		
			Intravenous	Intraperitoneal	Oral
Mouse	GFF	Female	29	240	760
Mouse	A2G	Female	Not tested	Not tested	660
Rat	WAG	Male	52	230	> 1100

Table 2.—The Effects of Serpajmaline on Weight Increase in Mice (A2G).

Group	Number in group	Group mean body weight (g.)													Per cent increase in body weight
		Weeks of treatment													
		Initial	1	2	3	4	5	6	7	8	9	10	11	12	
Test—Males ..	4*	8.5	13.1	17.4	20.1	20.4	22.0	23.3	24.5	24.3	26.5	27.8	26.6	27.3	222†
Control—Males ..	5	8.6	14.2	18.5	21.7	23.7	24.4	25.8	26.2	27.0	27.9	28.9	29.2	30.4	253
Test—Females ..	5	9.3	12.9	15.7	17.4	18.3	18.4	19.1	20.3	20.6	21.6	22.5	22.1	22.7	144†
Control—Females	5	9.1	13.8	16.9	18.9	19.6	19.4	20.9	21.2	21.8	22.5	23.4	23.3	24.0	164

* One animal died at the beginning of the experiment through faulty administration technique.

† The depressions in growth rate are significant $0.1 > P > 0.05$).

Table 3.—The Group Oxygen Consumption Rates of Mice after Daily Oral Dosing with Serpajmaline.

Group	Number in group	Group oxygen consumption rate litres/kg./hour						
		Days of treatment						
		15	16	17	20	21	85	86
Test—Males	4*	4.02	3·71	3·71	3.13	3.97	3.88	3.59
Control—Males	5	4.83	4.81	4.02	3.51	3.87	3.97	3.86
Test—Females	5	4.77	4.36	3.52	3.00	4.11	4.11	3.72
Control—Females	5	4.50	3.67	3–34	3.55	3.73	3.29	3.13

* See Table 2.

4 to 6 volts., (twice the threshold value determined at a stimulation rate of 180 per minute).

The normal heart rate and the maximum drive rate were determined immediately before and immediately after the auricles had been in contact with the drug for 20 minutes.

Results

STUDIES IN CONSCIOUS ANIMALS

1. *Acute Toxicity*

The results are given in Table 1.

In order to know whether the acute toxicity of serpajmaline changed on standing, the acute intravenous toxicity of a 0.3% w/v solution of serpajmaline was determined on female mice (GFF strain, 16 to 20 g.) immediately after preparation and after standing at room temperature (ca. 20°C.) for 3, 6, 24 and 168 hours.

There were no changes in the intravenous LD 50 during the seven days of the experiment, the values ranging from 27 to 29 mg./kg.

2. *Subacute Toxicity*

The group mean bodyweights obtained during the twelve weeks of treatment are given in Table 2 and the group oxygen consumption rates during the third week and at the end of treatment are shown in Table 3.

The group oxygen consumption rates of mice during and after treatment with serpajmaline do not differ from those of the untreated controls. Twelve weeks treatment with the serpajmaline slightly depressed ($0.1 > p > 0.05$) the growth rate in both the males and the females, but in no way affected fertility in males or females (Table 4).

3. *Effect on Barbiturate Activity in Mice*

The results together with those previously obtained in a similar experiment with reserpine are given in Table 5. It will be seen that serpajmaline had no effects on the activities of the two barbiturates in mice.

4. *Analgesic Activity in Mice*

The group mean reaction times after serp-

TABLE 5.—THE EFFECTS OF INTRAPERITONEAL INJECTIONS OF SERPAJMALINE OR RESERPINE ON BARBITURATE ACTIVITY IN MICE.

Time after injection	Serpajmaline (50 mg./kg.)				Reserpine (2 mg./kg.)			
	Hexobarbitone sleep time Mean ± S. D.* (mins.)		Barbitone induction-time Mean ± S. D.† (mins.)		Hexobarbitone sleep time Mean ± S. D.* (mins.)		Barbitone induction-time Mean ± S. D.† (mins.)	
	Test	Control	Test	Control	Test	Control	Test	Control
1 hour	21 ± 4	23 ± 3	16.9 ± 1.1	19.2 ± 2.6	33.4 ± 10.9	26.0 ± 5.6	8.0 ± 1.3	19.5 ± 2.9
2 hours	Not tested	..	Not tested	..	69.8 ± 28.8	26.0 ± 5.6	4.9 ± 0.7	19.5 ± 2.9
3 „	26 ± 4	23 ± 3	17.1 ± 2.3	19.2 ± 2.6	92.4 ± 21.0	23.2 ± 1.9	3.2 ± 0.9	19.5 ± 2.9
6 „	21 ± 2	23 ± 3	17.3 ± 1.3	19.2 ± 2.6	75.8 ± 11.7	22.3 ± 1.9	3.8 ± 1.3	20.0 ± 2.9
1 day	24 ± 4	25 ± 5	20.2 ± 1.5	19.2 ± 2.6	30.4 ± 6.6	24.8 ± 5.2	12.9 ± 5.0	19.2 ± 1.7
2 days	24 ± 5	25 ± 5	23.0 ± 2.4	24.4 ± 2.1	15.0 ± 3.4	16.6 ± 4.0	20.3 ± 2.1	18.3 ± 2.1
3 „	16 ± 3	25 ± 5	21.3 ± 1.4	22.5 ± 1.5	12.3 ± 3.3	22.3 ± 1.8	34.5 ± 3.6	17.7 ± 1.5
4 „	28 ± 5	25 ± 3	22.8 ± 2.6	20.5 ± 2.5	11.4 ± 2.7	24.8 ± 5.2	31.3 ± 8.4	18.1 ± 3.8
5 „	24 ± 2	25 ± 3	22.4 ± 1.3	21.7 ± 2.2	12.0 ± 3.2	16.6 ± 4.0	22.6 ± 2.6	18.3 ± 2.8
6 „	19 ± 5	25 ± 3	19.7 ± 1.2	20.5 ± 2.5	17.6 ± 6.1	23.0 ± 2.9	20.2 ± 2.3	19.0 ± 1.4
7 „	Not tested	..	21.3 ± 1.4	21.7 ± 2.2	22.2 ± 2.5	27.0 ± 7.0	19.0 ± 3.5	18.0 ± 3.8

* 5 mice per group. † 10 mice per group.

ajmaline together with the control times are given in Table 6.

It will be seen that serpajmaline had no analgesic action even at a dose level equivalent to almost half the LD 50 value. Results obtained in a similar experiment with pethidine hydrochloride are included for comparison.

5. Effect of Repeated Doses of Serpajmaline on Blood Pressure in Renal Hypertensive Rats

The results are illustrated in Fig. 1. There were no changes in response over the four days when doses between 6.25 and 25 mg./kg. were used. There may have been slight cumulation, however, at a dose level of 50 mg./kg.

6. Brain Serotonin-Releasing Activity

Shore, Pletscher, Tomich, Carlsson, Kuntzman and Brodie[16] have shown that reserpine injected intravenously into rabbits rapidly reduces the level of serotonin in the brain. The low level of brain serotonin persists for about 40 hours and is finally restored to its normal value after 5 or 6 days. In the rauwolfia group of alkaloids only the tranqu-

Table 4.—Results of Breeding Experiments.

Group	Number of pairs	Number of pairs having litters	Number of young born	Number of young reared
Test	5*	5*	27	21†
Control	5	5	26	21†

* One of the males served two females as only 4 test males were available.
† One whole litter killed at birth.

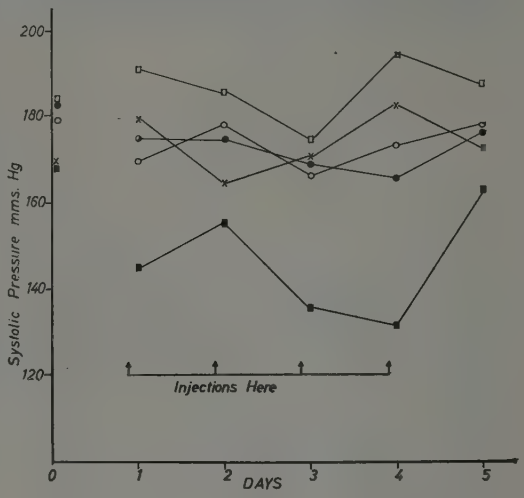

Fig. 1.—The effect of serpajmaline on systolic blood pressure in renal hypertensive rats. Intraperitoneal injections were given on four successive days and the blood pressure recorded one hour after each injection.

● Control animals.
□ 6.25 mg./kg. serpajmaline.
× 12.5 „ „
○ 25 „ „
■ 50 „ „

Table 6.—Tests for Analgesic Activity using Serpajmaline and Pethidine in Mice (all injections given intraperitoneally).

Drug	Dose mg./kg.	Mean reaction time ± S. D * (seconds)					
		Minutes after injecting drug					
		Initial	10	30	60	120	180
Serpajmaline ..	10	6 ± 2	7 ± 3	8 ± 3	7 ± 2	7 ± 2	7 ± 2
Serpajmaline ..	100	6 ± 2	11 ± 3	9 ± 3	10 ± 3	11 ± 3	10 ± 2
Pethidine	50	8 ± 2	43 ±17	28 ±15	17 ±10	Not tested	Not tested

* 10 mice per group.

illising members are able to release brain serotonin, and Brodie and his colleagues have suggested that these two activities may be associated. Hence it seemed of interest to ascertain the effect of serpajmaline on brain serotonin.

The results are given in Table 7. All results have been corrected for percentage recovery, known amounts of serotonin being added to normal rabbit brain, which was assayed simultaneously. These recoveries varied from 48 to 70%.

TABLE 7.—THE EFFECTS OF SERPAJMALINE AND RESERPINE ONE DAY AFTER ADMINISTRATION ON BRAIN-SEROTONIN LEVELS IN RABBITS (BOTH DRUGS INJECTED INTRAVENOUSLY).

Brain serotonin concentration (μg./g.)		
Controls	Serpajmaline (10 mg./kg.)	Reserpine (1 mg./kg.)
0.53	0.70	0.09
0.52	0.56	0.11
0.58	0.60	0.10
Average: 0.53	0.62	0.10

The normal serotonin concentration in rabbit brain is 0.52 to 0.58 μg./g. fresh tissue. Twenty-four hours after reserpine (1 mg./kg.) the level fell to 0.09 - 0.11 μg./g., but serpajmaline (10 mg./kg.) had no effect.

7. Effect on Blood Sugar Concentration

An intravenous injection of reserpine (5 mg./kg.) into the rabbit increased the blood sugar level within one hour of administration.[17] Serpajmaline had no effect on blood sugar when injected intravenously at 10 mg./kg.

8. Effect on Blood Clotting Time

Serpajmaline did not influence clotting time in the rabbit.

9. Neuromuscular-Blocking Activity in Chicks.

Intravenous injections into the chick of neuromuscular-blocking drugs produce characteristic patterns of muscular paralysis, which differ with the type of drug employed.[11] Thus "depolarising blockers" (e.g. decamethonium) produce a period of spasticity in the hind limbs and retraction of the head, whereas "competitive blockers" (e.g. *d*-tubocurarine) produce flaccid paralysis only. Both syndromes become apparent immediately after injection. Blocking agents of the third type, the anticholinesterases, produce a "depolarising block" resulting from the accumulation of of acetylcholine. The pattern of spasticity is similar to that observed with decamethonium, but there is a delay before onset, its length depending on the dose administered.

Intravenous injections of serpajmaline in doses from 2 to 4 mg./kg. were without effect. Higher doses (5 to 10 mg./kg.) produced slight ataxia, which was enhanced by covering the chick's eyes. Serpajmaline administered as a single intravenous dose of 15 mg./kg. was lethal to chicks.

Doses of decamethonium, *d*-tubocurarine and neostigmine required to produce characteristic neuromuscular block in the chick are 50, 500 and 1000 μg./kg. respectively.

Serpajmaline does not show neuromuscular-blocking or anticholinesterase activity in the chick.

10. Reserpine-like Activity in Kittens

Serpajmaline (50 mg./kg. orally) had no apparent effect on conscious kittens. There were no signs of gastric discomfort, diarrhoea, anorexia, sedation or relaxation of the nictitating membrane. Such responses are readily obtained with reserpine in doses as low as 0.5 mg./kg.

11. Tranquillising Effect in Monkeys

Within one hour of receiving reserpine, both monkeys became docile, approached the front of the cage and were obviously sedated. This condition was still apparent three hours later, but by 24 hours both had resumed their characteristically hostile attitude. Diarrhoea occurred in the monkey receiving reserpine by the intravenous route, but not in the one treated orally.

The two monkeys treated with serpajmaline remained hostile throughout the experiment. There were no signs of sedation or obvious discomfort in either animal. The serpajmaline did not appear to cause pain when injected into the femoral vein.

STUDIES IN ANAESTHETISED ANIMALS

1. Cats

Intravenous Injection.—Small doses of serpajmaline (0.25 to 2.0 mg./kg.) had no effect on

blood pressure, respiration, E.C.G. or the cardiovascular responses to adrenaline, noradrenaline, acetylcholine or histamine. The response of the nictitating membrane to pre-ganglionic stimulation remained unimpaired, but the fall in blood pressure and the bradycardia produced by peripheral vagal stimulation were completely abolished by serpajmaline in doses greater than 1 mg./kg. However, the apnoea resulting from central vagal stimulation remained unaffected. The vagal-blocking action of serpajmaline was reversible when doses of 1 or 2 mg./kg., were given, the response returning to normal within two hours.

The smallest dose of serpajmaline (0.25 mg./kg.) increased the tone and motility of the gut for about two minutes, and these effects increased in severity but not in duration with increasing dose.

With larger doses of serpajmaline (5 to 10 mg./kg.) a fall in blood pressure was produced; its degree and duration depended on the initial blood pressure level. In general, a fall of between 40 and 60 mms. Hg was obtained, the pressure returning to normal within 20 minutes. The fall in blood pressure was associated with depression of respiration, slight bradycardia, increase in the P-R interval of the E.C.G., reduction in the pressor response to noradrenaline and reversal of the pressor response to adrenaline. In addition, the reflex bradycardia normally obtained at the height of the noradrenaline response was abolished. The response of the nictitating membrane to adrenaline was reduced, and that to supra-maximal pre-ganglionic stimulation was sometimes reduced and sometimes unaffected (Fig.2). Peripheral vagal stimulation was blocked, but the apnoea resulting from central vagal stimulation remained unaltered. The blocking action of these doses on the effects of peripheral vagal stimulation was irreversible during the experimental period of four to five hours. The vascular responses to acetylcholine and histamine remained unchanged by serpajmaline in doses of 10 mg./kg.

Doses greater than 10 mg./kg. produced acute respiratory depression and death, when the heart remained in diastole.

Intravenous Infusion.—Most of the effects described above could be obtained by intravenously infusing serpajmaline at a rate of 0.5 mg./kg./minute. A complete block of the responses to peripheral vagal stimulation was obtained long before any effects on blood pressure became apparent (Fig.3). At this rate of infusion the responses of the nictitating membrane to preganglionic stimulation and to injected adrenaline were reduced by about 50 per cent. The blood pressure fell by about 40 mm.

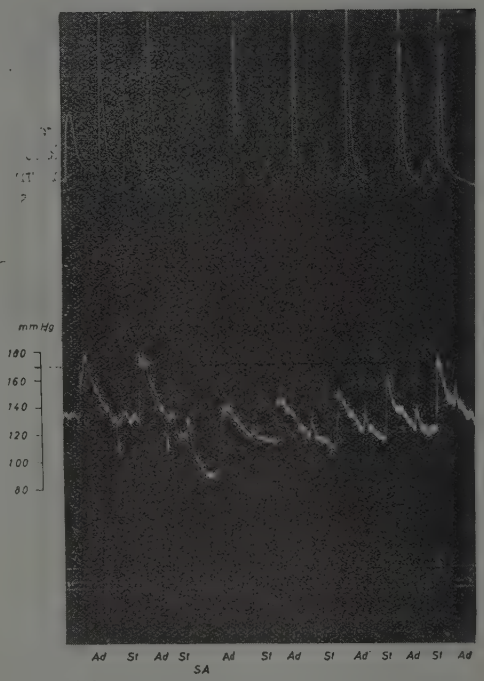

Fig. 2.—The effect of serpajmaline on the blood pressure and nictitating membrane of the anaesthetised cat (2.3 kg., chloralose 80 mg./kg. intraperitoneally). Intravenous injections of 10 μg. adrenaline at "Ad" and supramaximal stimulation of the vagosympathicus at "St". Serpajmaline, 8 mg./kg., injected intravenously at "SA".

Hg, bradycardia was observed, and the pressor response to noradrenaline was reduced and that to adrenaline was reversed. The depressor response to injected acetylcholine was not altered.

On stopping the infusion, the blood pressure rapidly returned to normal. A second infusion conducted at the same rate in the same animal produced a second fall in blood pressure, which was still obtained after cutting both vagi.

Respiration was not depressed with this rate of infusion, but with more rapid infusion rates some respiratory depression was observed. In a cat maintained under artificial respiration a total dose of serpajmaline of 100 mg./kg. infused at a rate of 1 mg./kg./minute was not fatal.

Injection Close to the Heart.—The fall in blood pressure and the bradycardia produced by peripheral vagal stimulation were completely blocked by

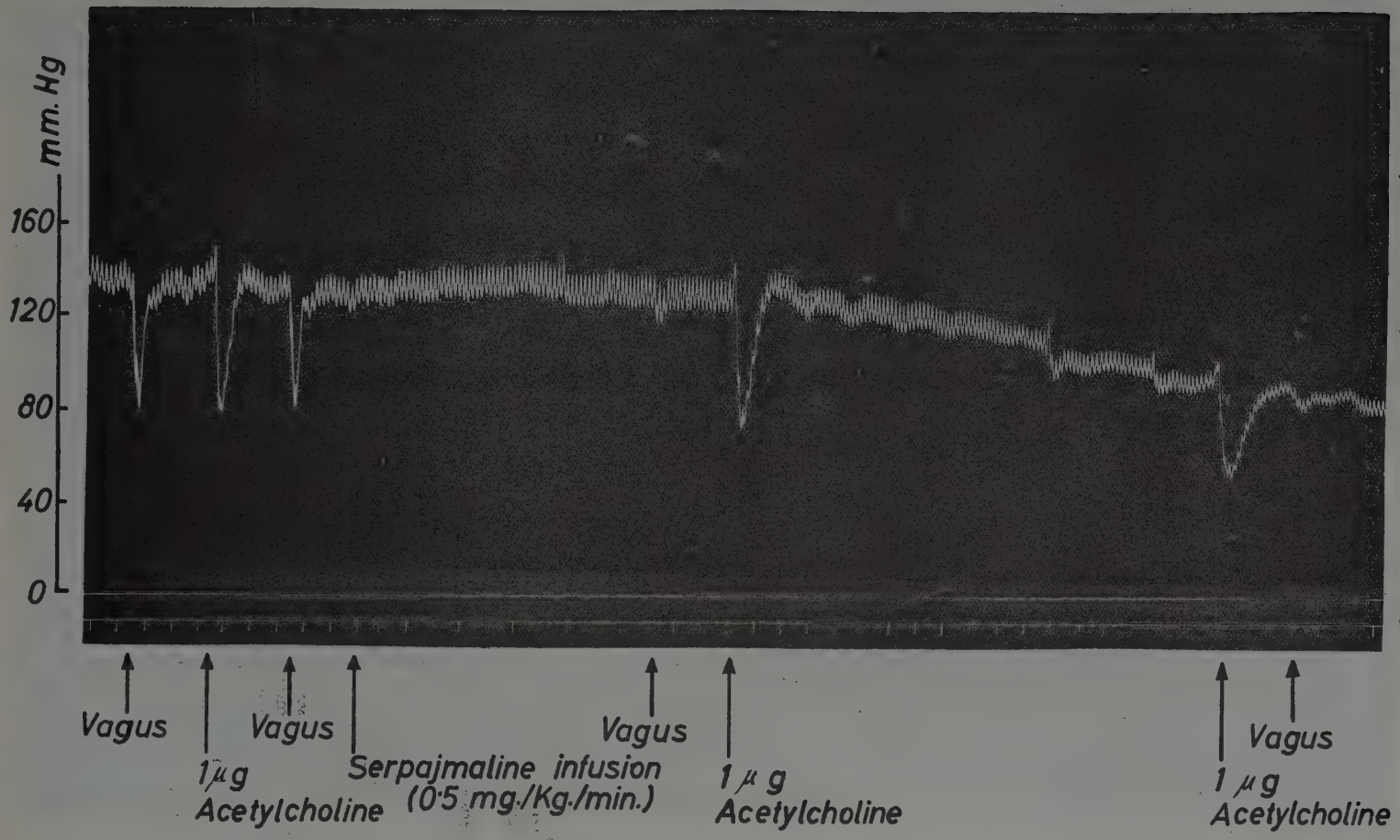

Fig. 3.—Effect of intravenous infusion of serpajmaline on the blood pressure of the anaesthetised cat (3.0 kg., chloralose 80 mg./kg. intraperitoneally). Vagus stimulated with supramaximal stimuli at "V" and acetylcholine 1 μg. injected intravenously at "Ach". Serpajmaline infusion started at "SA" (0.5 mg./kg./minute).

injecting 50 μg. of serpajmaline close to the point where the great veins enter the right auricle.

Injection into the Lateral Cerebral Ventricle.—Doses from 50 to 400 μg. administered directly into the lateral cerebral ventricle had no pharmacological actions in anaesthetised cats. Under these conditions reserpine (25 to 50 μg.) depressed both respiration and blood pressure.

Injection Directly into the Duodenum or Administration via Oesophageal Catheter into the Stomach.—Administration of serpajmaline into either the stomach or the duodenum of anaesthetised cats produce a fall in blood pressure after a latent period of 15 to 30 minutes. The fall in pressure, although small (20 to 40 mms. Hg) was attributed to the drug and not to deterioration in the animal's condition, the fall being associated with a blockage of peripheral vagal stimulation and a reduction in the pressor responses to adrenaline and noradrenaline. The drug did not depress respiration or alter the responses of the nictitating membrane to adrenaline or to supra-maximal pre-ganglionic stimulation. In one or two experiments the blood pressure returned to the pre-injection level, but usually it was still depressed at the end of the experiment.

Serpajmaline was not very active by the oral or intraduodenal routes, a dose 50 to 100 mg./kg. being required to produce pharmacological effects.

In two cats 100 mg./kg. administered into the stomach produced a rise in blood pressure. In the remaining six cats so treated this dose of serpajmaline was hypotensive.

2. *Dogs*

Intravenous Injection.—Doses of serpajmaline less than 5 mg./kg. did not lower the blood pressure of the anaesthetised dog. A complete block of peripheral vagal stimulation was obtained with 2 mg./kg.

Doses between 5 and 10 mg./kg. produced falls in blood pressure between 50 and 70 mms. Hg and lasting for 30 minutes. The fall in pressure was associated with reduced pressor responses to noradrenaline, reversed adrenaline responses and a depression of respiration. The fall in blood pressure produced by acetylcholine was unchanged.

Serpajmaline in doses of 20 mg./kg. produced a long lasting fall (100 mms.Hg) in blood pressure. The respiration was completely depressed, but normal respiration was again apparent after a short period of artificial respiration (2 to 5 minutes). The blood pressure remained depressed throughout the experiment.

Intraduodenal Injection.—Serpajmaline in doses less than 10 mg./kg. had no effect when administered intraduodenally into anaesthetised dogs. Doses of 10 or 20 mg./kg. produced falls in blood pressure (about 30 mms. Hg) after a latent period of 15 minutes. The depressor effect lasted from one to two hours.

3. *Monkeys*

Doses of serpajmaline less than 2 mg./kg. were without effect, but 2 or 4 mg./kg. produced a small fall in blood pressure and bradycardia, and blocked the effects of peripheral vagal stimulation. Higher doses (5 to 10 mg./kg.) produced a fall in pressure of approximately 50 mms. Hg, which lasted for 30 minutes. The pressor response to noradrenaline was reduced; that to adrenaline was blocked but not reversed.

Fifty mg./kg. given in divided doses (5, 10, 15 and 20 mg./kg.) at five minute intervals produced a sustained fall in blood pressure lasting for more than two hours, partial recovery occurring during the third hour. In this monkey the fall in pressure was associated with slight respiratory depression.

In similar experiments a single dose of reserpine of 5 mg./kg. produced a sustained fall in blood pressure (70 to 80 mms. Hg) lasting throughout the experimental period of 3 to 4 hours. Respiration in all three monkeys injected with reserpine was depressed, but no reductions in the pressor responses to adrenaline and noradrenaline were observed, and the effects of peripheral vagal stimulation were not blocked.

4. *Rabbits*

Serpajmaline has a smaller depressor activity in rabbits than in other species. Five mg./kg. produced only a transient fall in blood pressure; a dose of 10 mg./kg. was lethal.

Twenty mg./kg. administered in four doses of 5 mg./kg. at five minute intervals produced a fall in blood pressure of 50 mms. Hg.

Studies on Isolated Tissues

Guinea-pig Ileum.—At concentrations less than 10^{-5} serpajmaline had no effect on guinea-pig ileum, neither did it antagonise the actions of barium chloride or acetylcholine. At higher concentrations (1.3 to 1.6×10^{-5}) it increased the spontaneous activity of the ileum, but depressed contractions induced by barium chloride or acetylcholine. However, increased sensitivities to both barium chloride and acetylcholine were apparent after

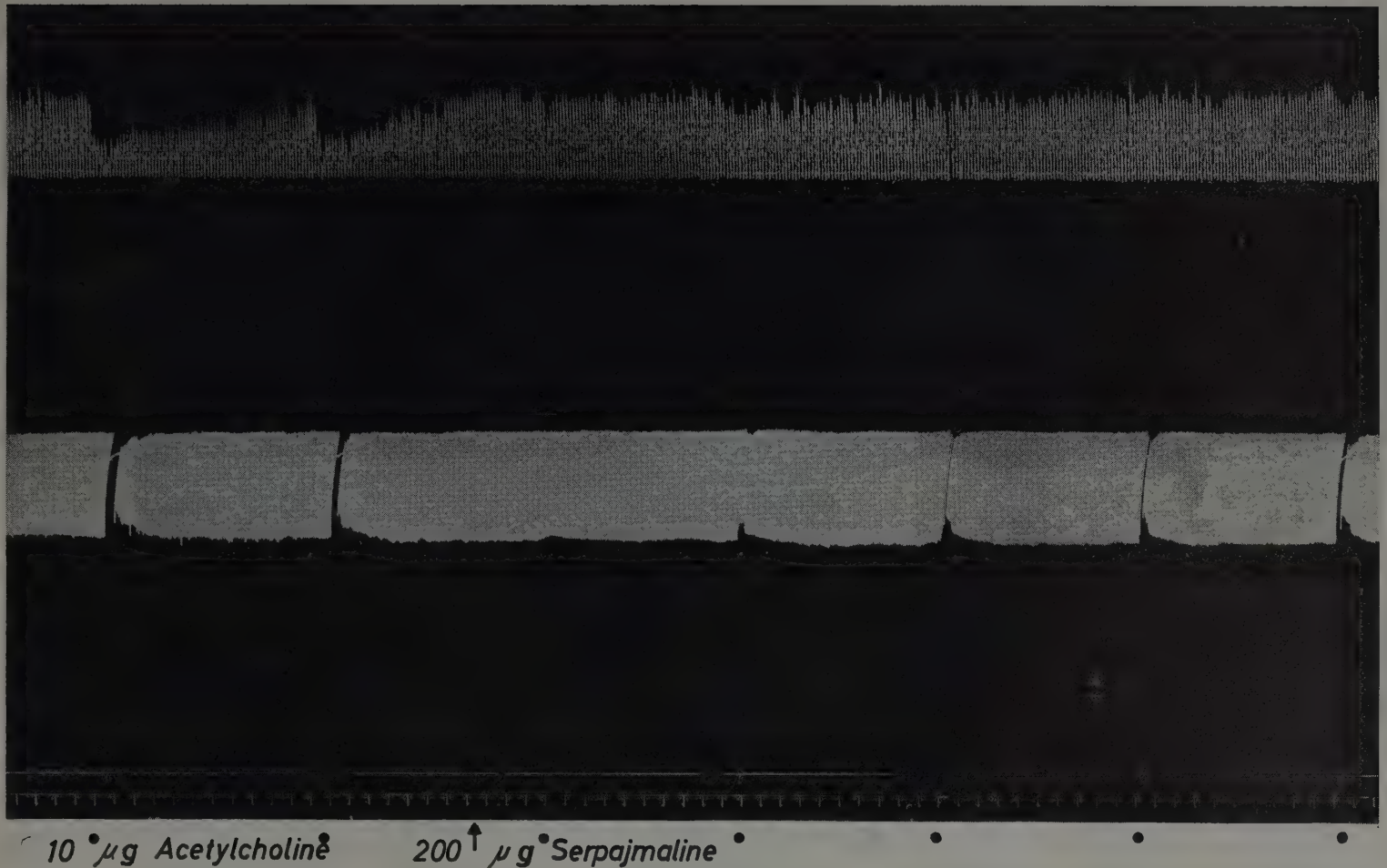

Fig. 4 :—Effect of serpajmaline on the response of the isolated perfused rabbit heart to acetylcholine. Upper tracing: coronory inflow. A decrease in coronory inflow is recorded as a decreased height of tracing. Lower tracing: Amplitude of beat. At 10 µg. of acetylcholine injected into perfusion fluid. At SA 200 µg. serpajmaline injected into perfusion fluid.

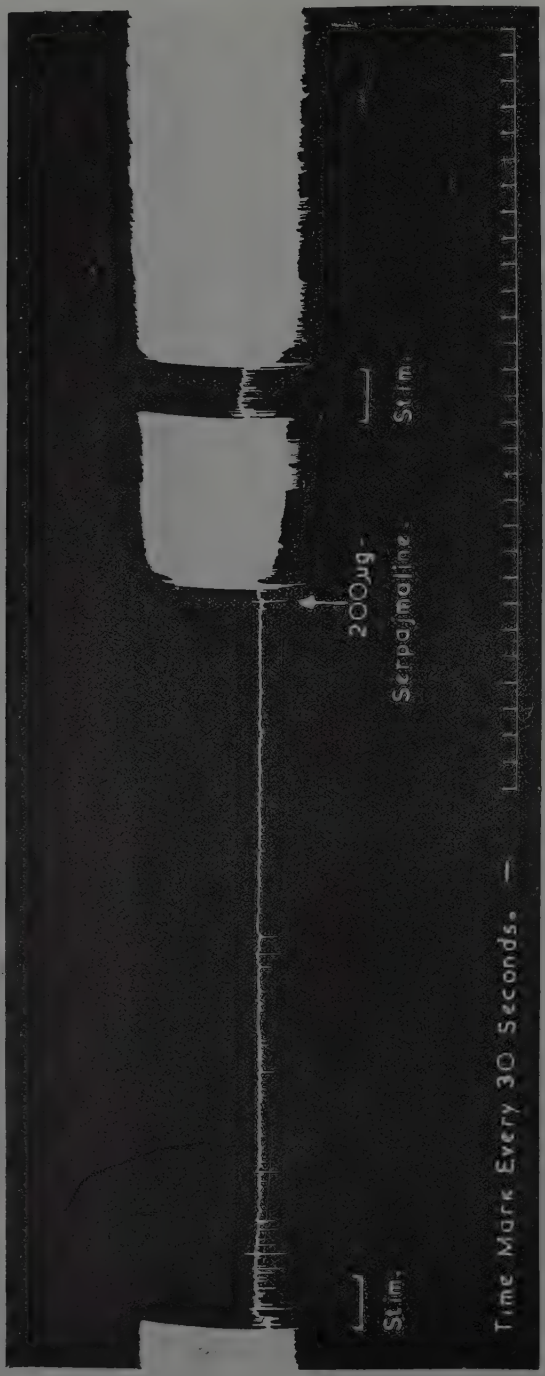

Fig. 5.—Effect of serpajmaline on the isolated perfused fibrillating rabbit heart. At the vertical arrow 200 µg. serpajmaline injected into the perfusion fluid. At "Stim" electrical stimulation applied to the ventricle.

washing the ileum several times with fresh Tyrode solution.

Rabbit Duodenum

Serpajmaline at concentrations of 1.5×10^{-5} did not affect normal peristalsis of rabbit duodenum.

Non-oestrus Rat Uterus

Concentrations of serpajmaline less than 1.5×10^{-5} had no effect on the non-oestrus rat uterus. Higher concentrations (2.5 to 5.0×10^{-5}) immediately produced alternate rhythmic contractions and relaxations of the uterus, which were maintained as long as the serpajmaline remained in contact with it. After washing out the serpajmaline marked increases in the sensitivity of the uterus to the stimulant actions of both carbachol and posterior pituitary extract were observed.

Chick Semispinalis Cervicis Muscle

Serpajmaline had no direct depolarising action on the isolated semispinalis cervicis muscle of the chick. At concentrations greater than 4×10^{-5} it antagonised the depolarising action of decamethonium: in this respect it was 500 times less active than *d*-tubocurarine.

Perfused Beating Rabbit Heart

Doses of serpajmaline less than 200 μg. had no effect on the isolated beating rabbit heart; 200 to 400 μg. slightly decreased the amplitude and reduced the coronary flow for about 3 minutes. The positive inotropic and chronotropic effects of adrenaline were unchanged, but the bradycardia normally produced by acetylcholine was completely abolished and did not return to normal for at least one hour (Fig.4).

Higher doses of serpajmaline (500 to 1000 μg.) produced bradycardia and marked decreases in amplitude and coronary flow.

Perfused Fibrillating Rabbit Heart

Serpajmaline injected into hearts in which fibrillation had been maintained for 15 minutes restored the beat to normal within one or two minutes (Fig.5). The dose required to produce this effect ranged between 100 μg. and 1000 μg., the mean value for 29 hearts being 350 μg.

A qualitatively similar result was obtained with quinidine (mean value 425 μg., range 200 to 800 μg. in 13 hearts).

Rabbit Auricles

Serpajmaline reduces both the normal rate of auricular beat and the maximum rate at which the auricle can respond to electrical stimulation (the effective refractory period). Qualitatively similar results were obtained with quinidine.

Discussion

The pharmacology of serpajmaline is complex, since it is a mixture of carbohydrate with at least five substances, of which three have been identified with known alkaloids.

These three alkaloids, serpentine, serpentinine and ajmaline, have been studied extensively by many workers, and there are wide differences of opinion about their sites and modes of action.

However it is generally agreed that all three reduce blood pressure in the anaesthetised cat and dog, that the fall in pressure is not affected by bilateral vagotomy and that none of the three compounds produces a fall in blood pressure when administered orally to the conscious dog. In addition, all three alkaloids stimulate intestinal musculature and depress respiration, and when injected in high doses into mice they produce convulsions. None of these substances is adrenolytic in the cat, but it is said that serpentinine possesses antiadrenaline activity and abolishes the effects of peripheral vagal stimulation in the anaesthetised dog.

Serpentine possesses slight ganglion blocking activity in the cat, the other two alkaloids being inactive in this respect. Ajmaline is reported to produce a slight rise in the fibrillation threshold in cats, rabbits and frogs without affecting the refractory period or conduction time (for references see 18).

More recently Arora and Madan have investigated the anti-arrhythmic activity of both ajmaline and serpentine and have found both alkaloids more active in this respect than quinidine.[19]

Thus it may be assumed that, though certain pharmacological properties of serpajmaline may be due to its three known constituents, their combined activity cannot account for all its actions.

Serpajmaline, injected intravenously into anaesthetised animals, reduced blood pressure in all the species examined; the dog and the monkey being the most, and the rabbit the least, sensitive of the animals tested.

The blood pressure fall obtained with serpajmaline in the anaesthetised animal is of short duration and is associated with

1. bradycardia
2. block of cardiac responses to peripheral vagal stimulation
3. block and sometimes reversal of the pressor response to adrenaline
4. reduction in the pressor response to noradrenaline
5. reduction in the response of the nictitating membrane to adrenaline
6. variable effect on the response of the nictitating membrane to supra-maximal pre-ganglionic stimulation
7. depression of respiration
8. stimulation of intestinal musculature.

Serpajmaline does not affect the vascular responses to acetylcholine or histamine, neither does it influence the apnoea produced by central vagal stimulation. No depression of blood pressure or respiration is obtained when serpajmaline is injected directly into the lateral cerebral ventricle.

This evidence, and the results of the experiments conducted on conscious animals, suggest that serpajmaline does not depress the central nervous system and has little or no action at the ganglia of the autonomic nervous system. Presumably the fall in pressure produced in anaesthetised animals is more peripheral in origin.

However the fall in blood pressure cannot always be correlated with the anti-adrenaline activity, and it is possible that in some animals serpajmaline produces direct vasodilation.

The cardiac actions of serpajmaline in anaesthetised animals seem to us particularly interesting. Serpajmaline produces bradycardia and yet simultaneously blocks the fall in blood pressure and bradycardia produced by stimulating the peripheral end of the vagus. The bradycardia cannot therefore arise from stimulation of the parasympathetic system or be due to increased sensitivity of the sino-auricular node to normal parasympathetic impulses. It is unlikely that the bradycardia results from decreased sympathetic activity alone, since such a mode of action requires the existence of an intact parasympathetic system.

The results of the experiments on anaesthetised animals and on the isolated perfused rabbit heart preparation indicate that serpajmaline blocks the cardiac actions of acetylcholine without affecting its vascular actions; in this respect it has many similarities with quinidine. Thus many of the actions of serpajmaline in anaesthetised animals may also be obtained with quinidine itself.

Intravenous injections of quinidine sulphate (5 to 10 mg./kg.) in the cat produce a slight short-lasting fall in blood pressure, bradycardia, respiratory depression, blocking of the cardiac actions of peripheral vagal stimulation and reduction in the pressor responses to both adrenaline and noradrenaline, the vascular response to acetylcholine remaining unaltered.

The results obtained with serpajmaline on fibrillating rabbit hearts and on electrically driven auricles show that serpajmaline is more active than quinidine.

Conclusions

1. Serpajmaline is not toxic when administered by the oral route to mice and rats, the LD 50 values being 750 and >1100 mg./kg. respectively.

2. The oxygen consumption rate of mice receiving a daily oral dose of serpajmaline (125 mg./kg.) is not affected after twelve weeks' treatment.

3. The growth rate of mice is slightly depressed ($0.1 > p > 0.05$) after twelve weeks of daily oral dosing with serpajmaline (125 mg./kg.).

4. Serpajmaline is much less active in the renal hypertensive rat, and in anaesthetised animals generally, than it is in the conscious dog.

5. In the anaesthetised animal serpajmaline reduces blood pressure in several ways, of which the most important is probably its anti-adrenaline activity. In some animals a direct vaso-dilator action may occur. Serpajmaline does not possess ganglion-blocking, atropine-like or anti-histamine activity.

6. In conscious mice, rats, rabbits, cats, monkeys, and chicks, serpajmaline has no central sedative, analgesic, neuromuscular-blocking, or anti-cholinesterase activities. It does not affect the activity of barbiturates in mice or the level of serotonin in the brain, the blood sugar concentration or the clotting time in the rabbit.

7. Serpajmaline possesses many of the actions of quinidine, the principal one being a potent antifibrillatory action on the isolated perfused mammalian heart.

Acknowledgements

We express our gratitude to Mr. B. Basil, who performed the experiments on renal hypertensive rats ; to Miss Patricia Sutherland for her work on perfused rabbit hearts ; to Mr. A.J. Clarke who investigated the action of serpajmaline on the isolated rabbit auricles and to Dr. Sarfaraz Siddiqui for assisting in some of the experiments. We are also indebted to Dr. Salimuzzaman Siddiqui for a generous supply of serpajmaline.

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ANTIFIBRILLANT ACTION OF THE SERPAJMALINE COMPLEX AND ITS CONSTITUENT ALKALOIDS

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In the preceding communication the pharmacological action of the alkaloidal complex serpajmaline,[3,9,10] isolated by S. Siddiqui, [23,24] from the fresh undried roots of *Rauwolfia serpentina* has been described. Serpajmaline, which is free from reserpine and other weaker bases, has a hypotensive action without central side effects. With reference to the antifibrillant cardiac action of serpajmaline,[3] it was considered of interest to study the action of the various alkaloidal and non-alkaloidal constituents of the complex, and the present paper deals with the results obtained from this study.

The fraction serpajmaline includes ajmaline, serpentine and serpentinine, two unknown compounds and a carbohydrate-like substance. Ajmaline belongs to the tertiary indoline alkaloids; serpentine and serpentinine belong to the quarternary and hydronium bases. The three alkaloids ajmaline, serpentine and serpentinine were isolated by S. Siddiqui and R. H. Siddiqui.[20,21,22] These alkaloids have been studied extensively by many workers and there are wide differences of opinion regarding their sites and modes of action. It is generally agreed that serpentine reduces blood pressure,[14,16,11,7,19] is a more active substance than ajmaline[13] and more toxic than ajmaline and serpentinine.[5] Its hypotensive activity is probably due to acute vasodilatation.[19] The pressor effect of adrenaline was reduced to about the same extent as after equal doses of ajmaline.[2] There is a discrepancy in the description of the blood pressure effect of serpentinine and ajmaline. Chopra and coworkers [5,6,11] observed that ajmaline and serpentinine raised the blood pressure, while other workers found that both alkaloids have a weak hypotensive action [1,15,16,17] and do not contribute much to the hypotensive activity of an alkaloidal mixture.

Method

The importance of potassium concentration for the occurrence of heart fibrillation became evident both for auricular and ventricular fibrillation. The fibrillation occurs when the potassium concentration of the solution is reduced.[12]

Isolated Rabbit Heart (Langendorff method).—After fixing the electrodes on the tip and the base of the heart, ventricular fibrillation was produced by electric stimulus: stimulation for 2 to 4 minutes, frequency 200-400, 5-10 mA, sharp rectangular impulses. If the fibrillation was not arrested by itself within 5 minutes after that, the fibrillation lasted for a longer period (40-60 minutes). After 10 minutes fibrillation a control solution was injected and 5 minutes later a solution of the drug was introduced via a polythene cannula opening near the coronary arteries to the perfusion fluid.

Perfusion Fluid.—McEwen solution with lower potassium chloride content and without sodium dihydrogen phosphate: sodium chloride, 7.6 g.; potassium chloride, 0.24 g.; calcium chloride, 0.19 g.; sodium bicarbonate, 2.1 g.; dextrose, 2.0 g.; sucrose, 4.5 g.; aqua dest., 1000 ml.

Isolated Dog Heart.—After perparing carotid artery and jugular vein of an anaesthetised dog (narcosis: chloralose 80 mg./kg. i.v., or pentothal 15 mg./kg. + phenobarbitone 25 mg./kg. i. v., and phenobarbitone 75 mg. / kg. intraperitoneal),

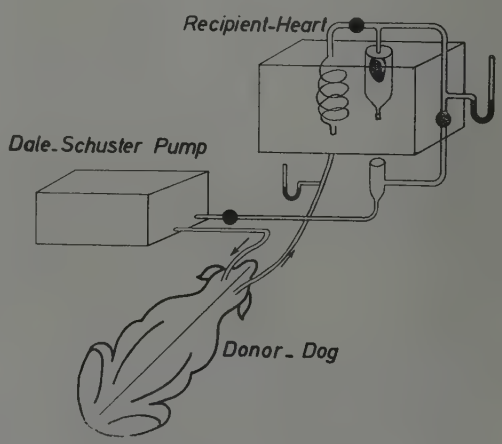

Fig. 1.—Arrangement of an isolated perfused dog heart connected with the circulation of second anaesthetised dog. The blood flow coming from the carotid artery passes: (1) mercury manometor; (2) spiral in isolated organ bath; (3) isolated dog heart; (4) blood reservoir; (5) Dale - Schuster pump; and (6) jugular vein. A second direct connection is between isolated heart and blood reservoir, passing a second mercury manometer registering the pressure in the aorta of the isolated heart.

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the aorta of an isolated dog heart is connected with the circulatory system of the anaesthetised dog between the carotid artery and the jugular vein (see Fig. 1). The blood then circulates from the carotid artery of the donor dog and passes a spiral of the organ bath (37 °C.) via a two way cannula either to the isolated heart through the coronaries, blood reservoir, Dale-Schuster pump and back to the jugular vein of the donor dog (=way I), or directly to the blood reservoir (=way II). Two mercury manometers are connected in this system, the one showing the blood pressure of the anaesthetised dog (donor dog), and the other the pressure in the recipient heart.

The preparation is done in the following manner:

(1) The two cannulas of the system are connected with the carotid artery and the jugular vein. The whole system is closed, except for the blood pressure manometer which is kept open.

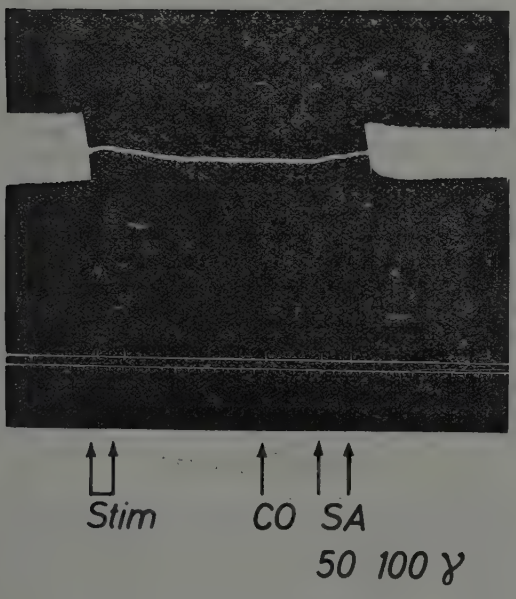

Fig. 2.—The effect of serpajmaline (SA) on a fibrillating rabbit heart after electrical stimulation (STIM). Thirteen minutes after fibrillation has started, a control solution (CO) was injected, afterwards serpajmaline was given, 100 γ was effective. Above: isolated heart. Below: signal and time (1 min.).

(2) The way II of the recipient system is then opened slowly to the extent the Dale-Schuster pump 1.5 ml. at a frequency of 80/min. can pump away by steady level in the blood reservoir. (3) After 10 minutes, the isolated heart (recipient heart) is prepared and immediately connected with the heart cannula. As the way I through the recipient heart is opened, the way II is simultaneously reduced to bring the pressure in the aorta of the recipient heart to 50-60 mm. Hg.

The recipient heart is connected with an isometric writing lever. Fibrillation is induced in the perfused beating recipient heart by electrically stimulating the ventricle. Fibrillation always appeared after short stimulation (10-30 seconds). Frequency, 200-700/sec.; duration, 0.75 msec.; 1-10 mA.; stimulus, sharp rectangular. The fibrillation persisted for periods of 60-120 min.

Results

As already described[3] serpajmaline arrested the fibrillation of the isolated rabbit heart immediately after the injection of 100-300 γ (see Fig. 2), average value 182 γ (14 hearts). Repeated attemps to reinduce fibrillation by electrical stimulation during the first hour after the injection of serpajmaline were unsuccessful.

Following the technique described above, fibrillation was induced in the isolated dog heart by electric stimulus, and about 10 minutes later a solution of serpajmaline (4 mg./kg.) was injected into the V. femoralis of the donor dog. After a

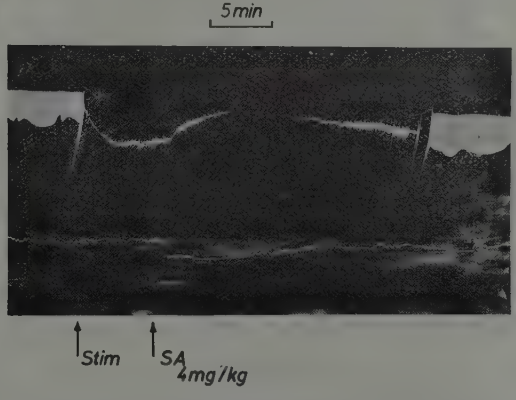

Fig. 3.—The effect of serpajmaline on the fibrillating isolated dog heart injected intravenously to the donor dog. The isolated dog's heart is connected with the circulation of a second anaesthetised dog. The isolated dog heart was electrically stimulated (STIM) till fibrillation appeared. Seven minutes after fibrillation started, serpajmaline (SA) 4 mg./kg. was injected intravenously to the donor dog. Twenty minutes after injection the fibrillation of the isolated heart was arrested. Above: isolated dog heart (recipient). Below: blood pressure in the system.

latent period of 20-25 minutes the fibrillation was completely arrested. The latent period may be taken as the time the drug takes to flow into the recipient heart after passing the artificial circulatory system. This finding shows that serpajmaline exercises its antifibrillant action well within the range of therapeutic dosage (see Fig. 3).

During a few of these experiments the recipient heart showed irregularity and partial block on its own without any artificial manipulation. After injection of 2 mg. serpajmaline into the heart via cannula, the heart in such cases recovered immediately and the rhythm was normalised.

It was of further interest to clear up the question if any of the alkaloids in serpajmaline is responsible for the heart action. The following bases and combinations have been investigated: (1) the total basic fraction of the serpajmaline complex; (2) the non-basic fraction of serpajmaline containing sugars and sugar acids and chlorides; (3) the alkaloids: ajmaline, serpentine and serpentinine; and (4) serpajmaline with the addition of 10-20% of ajmaline.

The following results were obtained on the isolated rabbit heart:

The basic fraction of serpajmaline showed an antifibrillant action with 50 to 100 γ (8 hearts).

The non-basic fraction of serpajmaline arrested fibrillation in an average dose of 217γ (ranging 100-300 γ) (6 hearts).

Ajmaline in an average dose of 63 γ, ranging 25-100 γ (8 hearts), and serpentine in an average dose of 200 γ (9 hearts) have both got an antifibrillant action. Serpentinine was ineffective even in doses of 600γ. K. J. Child[4] could confirm the results with ajmaline, serpentine and serpentinine. He also found ajmaline and serpentine to exercise an antifibrillant action on the isolated auricle.

In experiments with serpajmaline with added ajmaline it was observed that this combination arrests fibrillation in an average dose of 26 γ ranging between 6.125 and 75γ (16 hearts).

Discussion

Recently it was found that the combined activity of the known alkaloidal constituents of serpajmaline, (ajmaline, serpentine and serpentinine) cannot account for all its actions.[3] In the present investigation also it has been observed that the antifibrillant action of serpajmaline is not only due to ajmaline and serpentine, as its non-basic fraction has this action too. Ajmaline is 2-3 times more active than serpajmaline. Serpentine acts quantitatively similar to serpajmaline. The alkaloid content of serpajmaline is 35 to 40% depending upon the age of the root. From the results obtained so far on the chemical side of investigations, it would appear that, if ajmaline and serpentine only were to be responsible for the antifibrillant action, this action should be still stronger in comparison with that of serpajmaline. It has moreover been shown that the non-basic fraction of serpajmaline has likewise an antifibrillant action in a dose of 100 to 200γ , and the action of serpajmaline is intensified with the incorporation of 20% ajmaline. In fact this combination arrests fibrillation in most cases with a dose of 6.125 to 12.25 γ as against 182γ of serpajmaline, and this intensification of action cannot be accounted for on a merely addition basis.

Conclusion

Serpajmaline and the alkaloids ajmaline and serpentine contained in this complex have an antifibrillant action on the isolated rabbit heart. Ajmaline is more active than serpentine. Serpentinine is inactive.

The heart action of serpajmaline does not depend only on its alkaloidal content ajmaline and serpentine.

The non-basic fraction and the basic fraction of serpajmaline have both an antifibrillant action on the isolated perfused rabbit heart. The basic fraction was more active than the non-basic fraction of serpajmaline.

The addition of ajmaline to serpajmaline strongly potentiates its antifibrillant action.

With a newly developed method for testing the heart action, which consists in connecting the isolated dog heart with the circulatory system of a second anaesthetised dog, it has been demonstrated that serpajmaline arrests the heart fibrillation in therapeutic dosage (4 mg./kg. intravenously).

Acknowledgement

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CULTURE AND PROPAGATION OF RAUWOLFIA SERPENTINA, BENTH. IN EAST PAKISTAN

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Status of Supplies

The world-wide reputation of *Rauwolfia serpentina* in the treatment of hypertension and mental ailments has brought in its train the question of commercial availability of the roots of the plant. So far the roots have been mostly collected from the natural wild growth of the plant, and Java and India have been the main supply sources of the drug. Due to heavy and irresponsible collection, however, it is reported that Java has been denuded of the plant, and the Balai Penjelidekan Thenik Pertanian Bogor are lately taking measures for its restoration in the island.

Rauwolfia serpentina also occurs in Ceylon, Burma, Siam, Sumatra, the Andaman Islands, and East Pakistan, but the roots obtained from these countries vary in the relative content of their individual and total alkaloids. Although this variability has some effect on the biological activity, the material from these countries definitely is usable provided it is properly identified.[1]

A. J. Feuell in his paper[2] on the genus *Rauwolfia* stated, that the future was likely to see a steady and probably increasing demand of *Rauwolfia* roots, and to meet this demand several lines of development were suggested, viz., (a) fostering of existing growths and cultivation in the districts where the plant is indigenous in order to supplement wild material, (b) exploitation of plants from areas hitherto untapped, (c) use of alternative species as sources of reserpine and possibly of other alkaloids, (d) chemical and pharmacological study of species not so far examined.

In India, the Forest Department have arranged to survey the Indian forests with reference to (a), but the results of the survey have not yet been published. In Pakistan also, a grant was sanctioned by the Pakistan Council of Scientific and Industrial Research to the Dacca University for the survey of medicinal plants including *Rauwolfia*. The result of this survey has not yet been published either. We are thus still very much in the dark about the extent of the sources of *Rauwolfia* roots for commercial exploitation in tonnage requirement from the wild natural growth of the plants.

Necessity of Cultivation

Although it may be possible for a country with sufficient natural growth of the plant to rely on this source, for high tonnage requirement and uniform supply of the material, recourse to planned cultivation of the *Rauwolfia* species would be desirable. Consequently, even in India, cultivation of the species has been started and reported yields vary from 1000 to 2000 lbs. per acre.

On the basis of published information on the culture of *Rauwolfia serpentina* and or other species, certain broad facts can be summarised as follows:- (i) Propagative materials are seeds, root cuttings and stem cuttings. (ii) Germination of the seeds varies from 25% to 40% , the fresh seeds giving better results than the old. (iii) A light well-drained soil with a top-dressing of farmyard manure provides the best conditions for cultivation. (iv) From its far flung geographical distribution and its manifest edaphic tolerance, one may expect *Rauwolfia* to lend itself readily to successful cultivation under a wide range of conditions.

The necessity for the cultivation of *Rauwolfia serpentina* in Pakistan has assumed increasing importance with recent researches on the serparation of more active substances from the root material.[3,4] *Rauwolfia serpentina* grows wild in East Pakistan, but with the increasing demand the natural sources will be rapidly depleted. The plants do not occur gregariously but are widely scattered. This would make for a high collection cost and also render plant liable to extinction in the course of a few years. To meet this difficulty and ensure regular supplies of a uniform quality, it would be necessary to encourage large scale cultivation of the species for commercial exploitation.

Experimental Cultivation in Pakistan

In view of the considerations referred to above, a scheme "Investigation into the Possibility of Culture of *Rauwolfia serpentina, Benth.*" was submitted by the author to the Pakistan Council of Scientific and Industrial Research in 1954. The scheme was sanctioned and is now in its fourth year. The results of investigations carried out in the experimental plantation under this scheme are summarised below:-

1. Experiments to study the yield of roots under three different spacings between plants, viz., (a) 1′×1′, (b) 1½′×1′, and (c) 2′×2′, have

shown that the spacing of $1\frac{1}{2}' \times 1'$ is most suitable for the cultivation of *Rauwlfia serpentina*, keeping in view the yield of roots and interculture operation. This spacing is now being practised here and is recommended to other growers of *Rauwolfia serpentina* plants. The spacing gives a yield of 1500 to 3000 lbs. of roots per acre.

2. For the vegetative propagation of the plants of *Rauwolfia serpentina*, stem, root and leaf cuttings have been experimented with. It has been found that while both stem and root cuttings can be used successfully for the vegetative propagation of the species, the leaf cutting has no propagative value. Five hundred and forty pieces of stem cuttings were planted in plot 4, on 8th and 9th June 1958. The cuttings sprouted but, due to an unexpected spell of drought, most of them died later on. Following this, another 300 pieces of stem cuttings were planted and started sprouting. Ultimately 30 cuttings established themselves into full-fledged plants, giving 10% success. In 1959, with the advent of the northwester (April), 625 stem cuttings were planted. Out of these cuttings 72 could establish themselves into full-fledged plants, giving 12% success. At the same time 10,000 root cuttings were planted, and it was observed that 2,771 sprouted and produced as many established plants, so that here the success was 36%. From our observations on the sprouting of the stem and root cuttings carried out so far, it may be concluded that root cuttings are better propagative material of the plant than the stem cuttings.

3. For the propagation of the plants of *Rauwolfia serpentina* with seeds, freshly ripened fruits, dried fruits and peeled seeds (i.e. the seeds in three different states) were used in order to determine their relative suitability. These experiments established that the peeled seeds are most suitable for propagation. They germinate quickly and the percentage germination of the seeds is also high. The peeled seeds are therefore now being used by us for the propagation of the plants.

Sowing Time for Optimum Germination

Germination tests on the seed of *Rauwolfia serpentina* have been carried out all round the year by monthly sowing to determine the most suitable time of the year which gives a maximum percentage germination of the seeds of this species. The results obtained so far are shown in the table. Allowing for variations due to non-determinable factors, it may be concluded from the data presented in this table that sowing in the period immediately preceding the monsoon (i.e. March and April) gives the lowest percentage germination, the optimum germination being obtained in the closing months of the monsoon with a record of 48% in July.

Date of sowing	No. of seeds sown	Date of germination	Time of germination*	No. of seedlings	Percentage
1.9.1957	1640	18.9.1957	17 days	225	14%
1.10.1957	500	18.10.1957	17 ,,	125	25%
1.11.1957	500	21.11.1957	20 ,,	120	24%
9.12.1957	200	31.1.1957	22 ,,	40	20%
13.1.1958	500	3.3.1958	53 ,,	100	20%
3.2.1958	400	13.3.1958	39 ,,	85	21%
1.3.1958	500	24.3.1958	23 ,,	42	8%
1.4.1958	500	3.5.1958	32 ,,	50	8%
1.5.1958	500	19.5.1958	18 ,,	72	14%
1.6.1958	500	21.6.1958	20 ,,	50	10%
1.7.1958	500	17.7.1958	16 ,,	240	48%
1.8.1958	500	15.8.1958	14 ,,	136	29%

*It may be noted that although a definite number of days has been given as 'the time of germination', it has been observed that the seeds continue to germinate for much longer periods, so much so, that even after an interval of a year, seedlings have been known to appear in the seedbeds.

Besides this trial, the seeds of *Rauwolfia serpentina* were also germinated for transplantation of seedlings. Four thousand nine hundred seeds of this species were sown in the seedbeds in April 1958 and were found to take 18 to 30 days for germination. The maximum and minimum percentages of germination in the various seedbeds were 16 and 5, respectively, while the average was 12. The corresponding data for percentage germination of 26,200 seeds sown in the month of May are: maximum 64% and minimum 13% with an average of 35%.

Further with the advent of the northwester and during the current monsoon, i.e. from May to July 1959, altogether 38,180 seeds were put to seedbeds for germination to obtain seedlings for transplantation in the fields used for the scheme "Large Scale Plantation of *Rauwolfia serpentina*." It was observed that during the month of May the time of germination was 13 days, the maximum germination was 45%, minimum was 37% and the average 41%. Similarly, during the month of June when 24,410 seeds were put to seedbeds, the maximum germination was 75%, the minimum 31% and the average 50%, and the time of germination 10 days. Again during the month of July when 7,200 seeds were put to seedbeds, the

maximum germination was 66%, the minimum 63%, and the average 65%, the time of germination being 12 days.

The above data from 1st September 1957 to 31st July 1959 shows that the germination of the seeds is rather erratic. This is because the process of germination was taking place under the natural conditions of moisture, temperature and aeration and other soil conditions existing at the time in the seedbeds. To obtain consistent values of germination percentage of the seeds, the germination must be conducted under uniform and controlled conditions of temperature, moisture, aeration and substratum, which can only be obtained in a modern type of "Scientific germinator".

Conclusions

These variations do not vitiate the main conclusion that the best time for obtaining seedlings of *Rauwolfia serpentina* is to start the seedbed sowing in the beginning of May, i. e. with the advent of the northwester, and continue the operation throughout the monsoon and even till October. Of course, the higher average percentage germination will be obtained during the four months of the monsoon, i. e. May, June, July and August.

As to the comparative efficiency of the propagative materials, it is concluded from our observation so far that the peeled seeds are the best material, and among cuttings, the root cuttings are better than the stem cuttings while leaf cuttings have no propagative value.

Some useful instructions for prospective cultivators are given in the Appendix.

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Appendix: Instructions for Cultivation

On the basis of experience gained in the course of investigations described in the paper, the following instructions are offered for the cultivation of *Rauwolfia serpentina* in East Pakistan and similar climatic areas.

(*i*) *Preparation of Seedbeds*

(a) The site of a seedbed should be so selected that the land is free from waterlogging and receives ample sunshine and air. A site having east and south ends open would be most suitable.

(b) The site should be cleaned and freed from grass roots and roots of other plants, and then dug up to a depth of at least 9″ by a 'kodali' or a shovel. Cow dung or farmyard manure and leaf mould should be mixed with the dug-out soil and the seedbeds prepared to fine tilth. In a seedbed of 10′×5′, one maund of the manure and one maund of leaf-mould should be mixed.

(*ii*) *Sowing of Seeds*

After the preparation of the seedbed, the seeds should be sown in lines 4″ apart between lines and 2″ apart in the line. The seeds should not be sown very deep, a depth of about 1/2″ being suitable. After sowing the seeds in the lines, the surface of the seedbed should be smoothed by hand. Thereafter, the beds should be copiously watered. During the monsoon, it may not be necessary to water the beds frequently, but if there is no rain for several days, the watering should be repeated. During the monsoon, the seeds should start germinating after a fortnight, but in the dry period and in the cold season, germination takes longer, some times as long as six weeks. Similarly, in monsoon the percentage of germination is very high going upto a maximum of 80%, but deteriorates as the season gets drier and colder. Usually one seed per hole is sown, and about 1 lb. of seed is needed for a seedbed of the dimensions given earlier.

(*iii*) *Care of the Seedbeds during Growth of the Seedlings*

Particular attention should be given to keep the seedbeds free from any weed, grass, etc., even after the seeds have germinated. It has been found that if a seedbed is not kept free from weeds, the germination is very poor, and the seedlings succumb to superior competition of grasses and weeds. It is essential for best results that the seedbed should be kept neat and clean from the very day of seed sowing till the seedlings are removed

for transplantation. Besides this care of the seedbeds, they should be watered whenever required, keeping in mind that they must never be allowed to get bone-dry and hard. The seedlings will be ready for transplantation when they are six weeks old and have six to eight leaves.

(*iv*) *Preparation of Site for Transplantation of the Seedlings*

As *Rauwolfia serpentina* cannot tolerate any waterlogging, the kind of field on which tea is grown should be ideal for *Rauwolfia serpentina* plants; it can be grown not only on valley land but also on the slope of the hill.

Before transplanting, the area to be transplanted with seedlings should be selected and prepared. It should be freed of weeds and grass and then one handful of mixed manure should be put in each hole. The mixed manure is prepared by thoroughly mixing one maund of rotten cow-dung, ½ maund of bone meal, 2½ seers of ammonium sulphate, and one maund of leaf mould, to be thoroughly mixed before application. After putting the mixed manure in each hole, the site will be ready for transplantation of the seedlings.

(*v*) *Uprooting the Seedlings (from the Seedbed) for Transplantation*

Before removing the seedlings, the seedbed should be watered so as to moisten the soil. The seedling should be removed not by 'pulling', but with the help of a 'niri' or 'khurpi', starting from one end of each line.

Starting from one end of the seedbed, the seedlings should be lifted gradually, one at a time, with minimum disturbance of the roots and keeping some lumps of soil adhering to the roots of the seedling in order to avoid injury to the roots. Seedlings thus lifted should be arranged on a tray. Care should be taken not to expose the seedlings to the strong light of the sun. If they are to be stored before planting, this storage should be in a cool, shady place, and if necessary they should be sprayed with water by mist sprayer. The best time for transplantation of seedling is early morning or late afternoon.

(*vi*) *Transplantation*

As the plant is small and has a weak stem, it has a sort of straggling habit. It has been found by experiment that the best method of planting is 1½′ apart between lines and 1′ apart in lines.

On the site selected for the plantation, lines 1½′ apart should therefore be drawn, preferably in the north to south direction. Then on each line holes should be dug one ft. apart. After the seedlings have been brought on the site of the transplantation field, individual seedlings should be placed and arranged one in each of the holes in the lines. Then the seedlings should be carried in the holes with the ball of the earth along with their roots up to the first pair of leaf from the ground and the soil round the root of seedlings tightly pressed. When transplantation is completed, the transplanted seedlings should be watered. After this, if there is a shower of rain each day or on alternate days, watering would not be necessary, but during a droughty period watering is essential.

It may be mentioned here that cloudy and drizzly days are preferred for the transplantation of seedlings. In such weather transplantation can be carried out throughout the day.

(*vii*) *Care after Transplantation*

The transplanted lines and especially the region around each plant should be kept free from weeds and grasses during the early stage of the growth of plants, and in order to give a neat and tidy appearance to the plantation, the space (about 1′) between plants in the lines should also be freed from untidy growths, especially during the early stage of growth of the plantation. Mulching of the root region of each line should be done once in a fortnight or in 3 weeks. Any gaps due to the withering of transplanted seedlings should be filled up by transplanting fresh seedlings. Generally it takes the seedlings 3-4 days to recover from the shock of transplantation, and within a week the plantation should look as established. As the plant grows in height, it will straggle due to the weak nature of the stem, and it will be necessary to put earth in the root region to make a ridge, the lines containing the plants would be ridges and the space between the lines would be furrows. The plants will start flowering when they are about 3 months old, and the flowering and fruiting would be continuous. For future plantation, the seeds (which are blackish when ripe) should be collected and seeds extracted regularly.

(*viii*) *Harvesting*

It has been found by experiments that the most economical harvesting age in respect of yield per acre and alkaloidal content is between 12 months and 18 months after transplantation. It has been estimated from our experimental plots that the yield of roots varies between 1500 lbs. and 3000 lbs. per acre.

It must be mentioned here that in the foregoing description seeds have been considered as the propagating material. However, *Rauwolfia* plants can also be propagated by root cuttings and stem cuttings, and therefore replantation of harvested plantations should not be a very difficult matter, at least in comparison to a fresh plantation, because in harvesting (during the process of digging and uprooting of plants) quite a number of ends of roots are bound to remain in the soil. These bits of roots will germinate and shoot out new plants as soon as they get suitable moisture and temperature conditions with the onset of the monsoon. Quite a number of ripe fruits will also drop underneath each plant and these too will germinate as soon as they get proper conditions for their germination. What will be necessary to complete a replantation is to fill up the gaps among these self-sown plants by fresh seedlings at the proper time and season.

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Rauwolfia serpentina and the drugs obtained from it and some of the other *Rauwolfia* species have now acquired high importance in the field of medicine. The present bibliography has been prepared as a compliment to the papers presented in this special issue. This bibliography incorporates the references previously published in Miscellaneous Publication No. 4 of the Pakistan Association for the Advancement of Science, compiled by A. R. Ghani, and includes over 2,000 references.

For the reader's convenience in searching the extensive literature in this field, the references are grouped under the following broad subject headings:

I. General, Botany and Pharmacognosy;

II. Chemistry;

III. Pharmacology and Therapeutics.

Within each section the references are arranged alphabetically according to the names of the authors.

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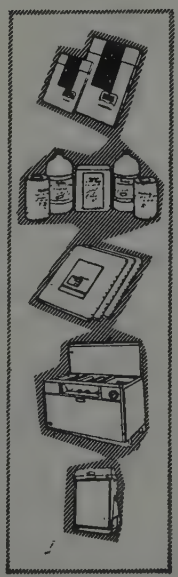

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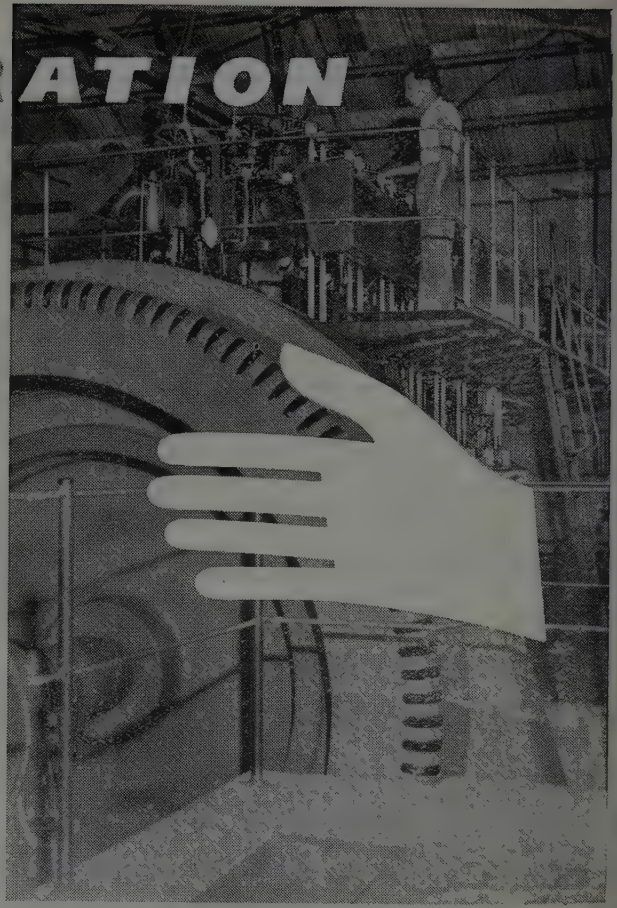

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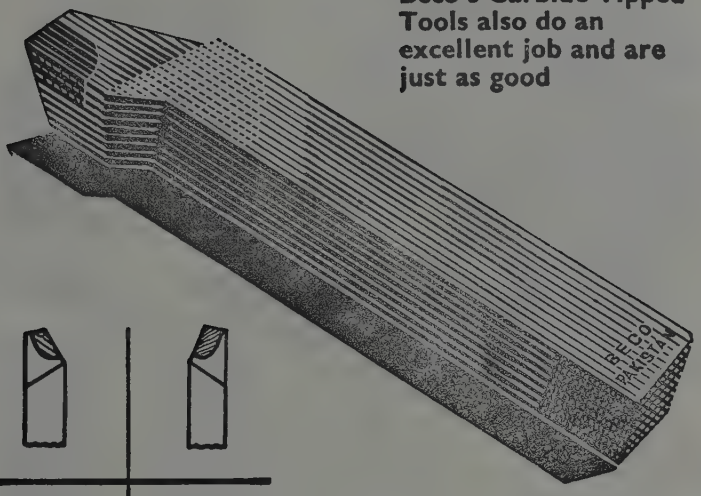

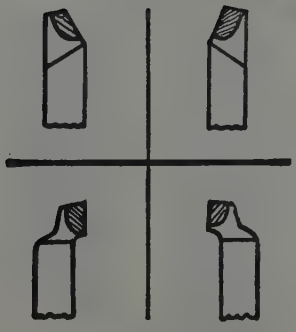

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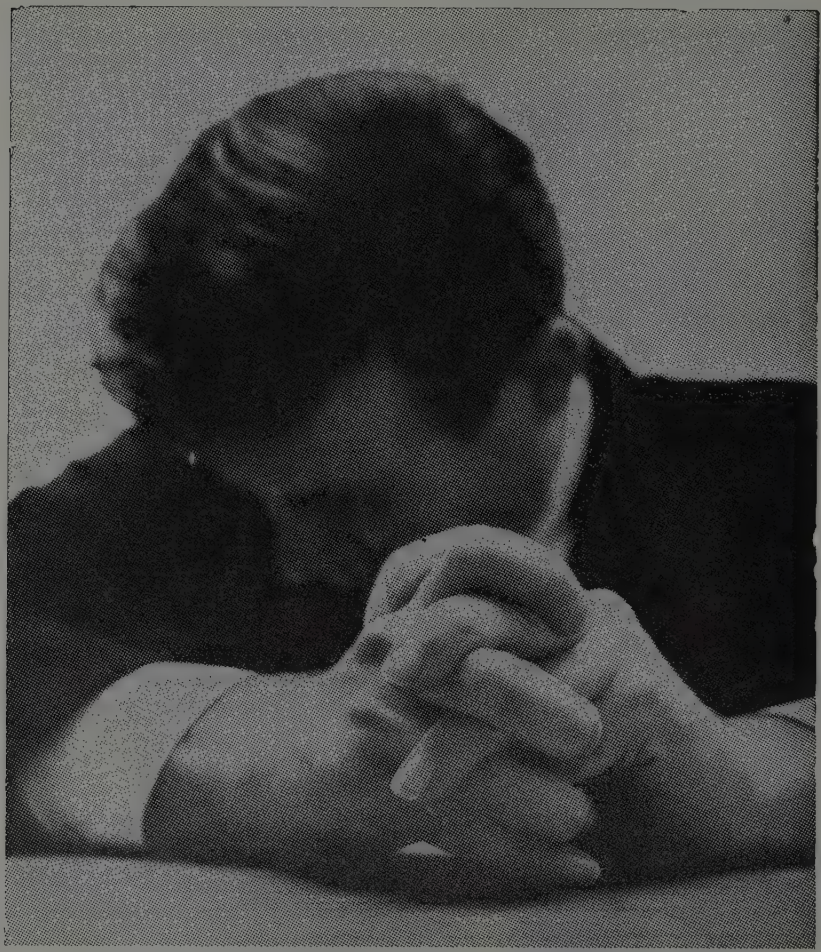

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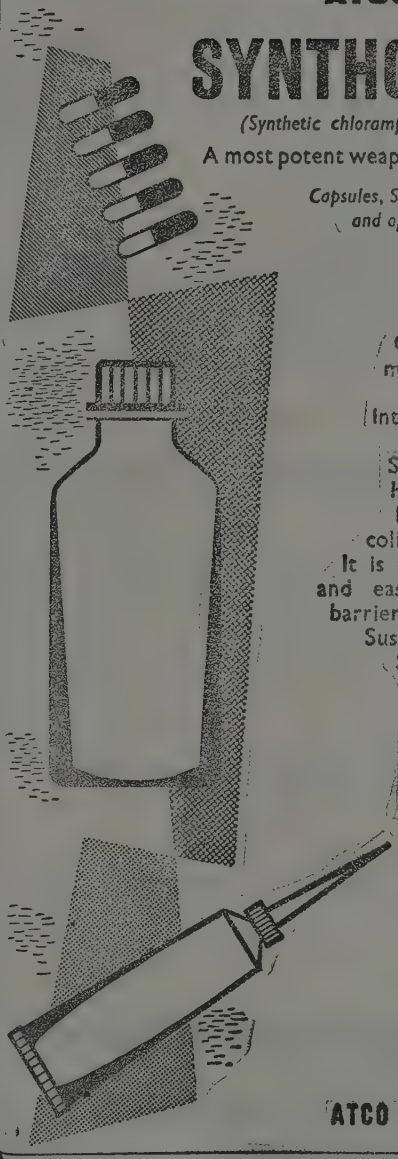

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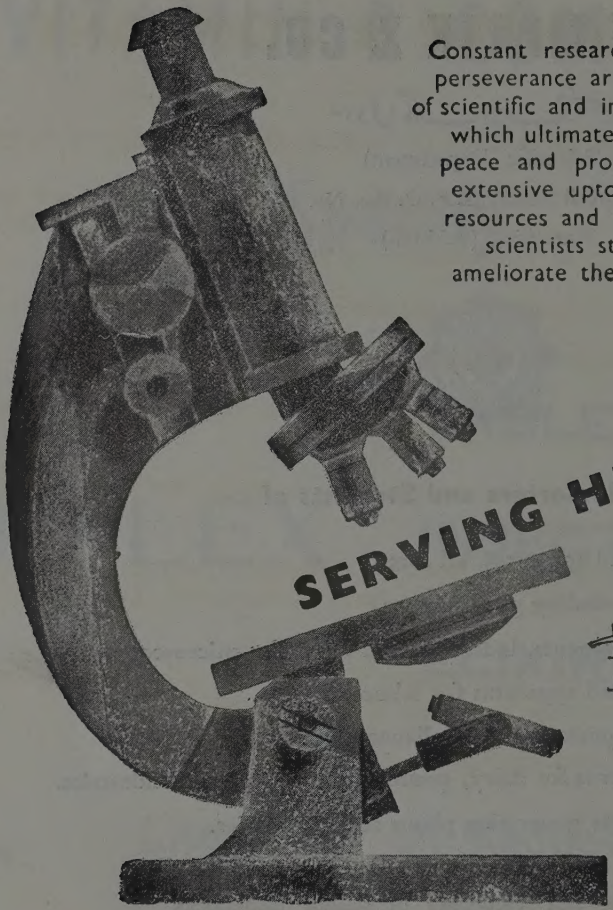

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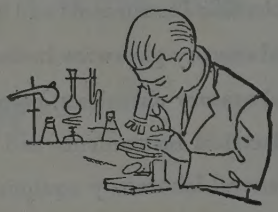

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